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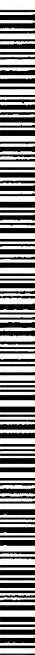
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(54) Title: METHODS AND COMPOSITIONS FOR THE TREATMENT OF CELL PROLIFERATION

(57) Abstract: The present invention relates to a use of a composition comprising one or more inhibitors of the citric acid, TCA, cycle, one or more inhibitors of glycolysis, and one or more inhibitors of oxidative phosphorylation for the manufacture of a medicament for the treatment of cellular proliferation, wherein said composition is administered to or in the region of the proliferating cells.

METHODS AND COMPOSITIONS FOR THE TREATMENT OF CELL PROLIFERATION**BACKGROUND TO THE INVENTION**

The treatment of conditions relating to cellular proliferation, malignant and benign, such as 5 tumours, hyperproliferative scars, cheloid scars, and restenotic processes at the level of a duct have several disadvantages, such as, for example, high toxicity, low efficacy, expense and the requirement for repeated or continuous administration.

10 The use of metabolic pathway inhibitors for the treatment of cellular proliferation is known in the prior art. For example, US 2003/30181393 describes inhibitors of glycolysis and oxidative phosphorylation; US 2003/0087961 described the use of inhibitors of glycolysis; EP 1372646, WO 02/072077, WO 2004/024676 described the use of glycolysis and transaminase inhibitors; US2002/0187534 and US2002/0024050 describe the blocking of fatty acid synthase to inhibit cellular proliferation.

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The present invention aims to overcome the problems of the prior art by providing alternative, improved treatments for cellular proliferation.

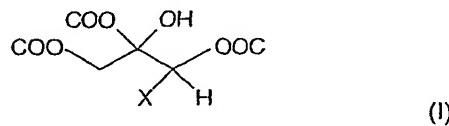
SUMMARY OF SOME EMBODIMENTS OF THE INVENTION

20 One embodiment of the invention is a composition comprising one or more inhibitors of the citric acid, TCA, cycle, one or more inhibitors of glycolysis, one or more inhibitors of oxidative phosphorylation and one or more slow release agents.

25 One embodiment of the invention is a composition as described above, wherein said TCA cycle inhibitor is an inhibitor of one or more of pyruvate dehydrogenase, citrate synthase, aconitase, isocitrate lyase, alpha-ketoglutarate dehydrogenase complex, succinyl CoA synthetase, succinate dehydrogenase, fumarase, malate synthase, glutaminase and pyruvate dehydrogenase complex.

30 Another embodiment of the invention is a composition as described above, wherein said TCA cycle inhibitor is any of arsenite, hypoglycin A, methylenecyclopropylacetic acid, alloxan, PNU, p-benzoquinone, fluoroacetate, halogenated acetates (iodo-, bromo-, chloro-acetate), halogenated acetyl-CoA (fluoroacetyl-CoA, bromoacetyl-CoA, chloroacetyl-CoA, iodoacetyl-CoA), halogenated crotonate (fluoro-, iodo-, bromo-, chloro-crotonate), 35 halogenated ketone bodies, (chloro-, fluoro-, bromo-, iodoaceto-acetate, fluoro-, chloro-,

- bromo-, iodo-butyrate, fluoro-, chloro-, bromo-, iodo-acetone), halogenated oleate (iodo, bromo, chloro, fluoro-oleate), halogenated citrate, halogenated citrate 2R, 3R isomer (fluoro-, bromo-, chloro-, iodo-citrate), dichlorovinyl-cysteine, halogenated aminoacids, malonate, pentachlorobutadienyl-cysteine, 2-bromohydroquinone, 3-nitropropionic acid, cis-crotonalide
- 5 fungicides, glu-hydroxyoxamate, p-chloromercuriphenylsulphonic acid, L-glutamate gamma-hydroxamate, p-chloromercuriphenylsulphonic acid, acivicin (alpha-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid, halogenated glutamine (fluoro, iodo, chloro, bromo-glutamine), or halogenated glutamate (fluoro, iodo, chloro, bromo-glutamate).
- 10 Another embodiment of the invention is a composition as described above, wherein said TCA cycle inhibitor is a compound of formula (I):

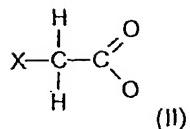


- 15 where X is halide, a sulfonate, a carboxylate, an alkoxide, an amine oxide or OH.

Another embodiment of the invention is a composition as described above, wherein formula (I):

- a halide is selected from the group consisting of: fluoride, bromide, chloride, and iodide,
- 20 - a sulfonate may be selected from the group consisting of: triflate, mesylate and tosylate,
- a carboxylate may be selected from the group consisting of: methoxylate and ethyloxylate,
- 25 - an alkoxide may be selected from the group consisting of: methoxide and ethoxide,
- an amine oxide is dimethylamine oxide, and
- where the stereochemistry is 2R, 3R,

- Another embodiment of the invention is a composition as described above, wherein said
30 TCA cycle inhibitor is a compound of formula (II):



where X is a halide, a sulfonate, a carboxylate, an alkoxide, an amine oxide, or an OH.

- 5 Another embodiment of the invention is a composition as described above, wherein formula (II):

- the halide is selected from the group consisting of: fluoride, bromide, chloride, and iodide,
- the sulfonate is selected from the group consisting of: triflate, mesylate and tosylate,
- the carboxylate is selected from the group consisting of: methoxylate and ethyloxylate,
- the alkoxide is selected from the group consisting of: methoxide and ethoxide, and
- the amine oxide is dimethylamine oxide.

15

Another embodiment of the invention is a composition as described above, wherein said TCA cycle inhibitor is any of

- p-benzoquinone, thiaminase, fluoroacetamide, halogenated ketone bodies, chloroacetoacetate, fluoroacetoacetate, fluorohydroxybutyrate, chlorohydroxybutyrate, 20 bromohydroxybutyrate), halogenated acetic acid, chloracetic acid, 6-diazo-5-oxo-L-norleucine (DON).

Another embodiment of the invention is a composition as described above, wherein said TCA cycle inhibitor is any of fluoroacetate, arsenite, acetoacetate, and betahydroxy butyrate.

25

Another embodiment of the invention is a composition as described above, wherein said TCA cycle inhibitor when comprising a halogen atom, comprises a halogen radioisotope in place of a stable halogen atom.

- 30 Another embodiment of the invention is a composition as described above, wherein said inhibitor of glycolysis inhibits at least one enzyme from the group consisting of hexokinase, glucokinase, phosphoglucose isomerase, phosphofructokinase, aldolase, triose phosphate

isomerase, glyceraldehyde 3-phosphate dehydrogenase, phosphoglycerate kinase, phosphoglyceromutase, enolase, pyruvate kinase, and lactate dehydrogenase.

Another embodiment of the invention is a composition as described above, wherein said inhibitor of glycolysis is a hexose sugar modified by removal of the hydroxyl group or by the substitution of the hydroxyl group with halogen atom or thiol at:

- C6,
- C1 or C2 or C5,
- C3 and/or C4, and/or
- 10 - C2 or C3.

Another embodiment of the invention is a composition as described above, wherein said inhibitor of glycolysis is any of 6-deoxy-6-fluoro-D-glucose, 6-deoxy-6-bromo-D-glucose, 6-deoxy-6-chloro-D-glucose, 6-O-methyl-D-glucose, 6-thio-D-glucose, 6-deoxy-D-glucose, C-6 modified or blocked derivatives of other hexose ring pyranoses, mannopyranoses, galactopyranoses, 6-deoxy-6-fluoro-D-glucose, 6-deoxy-6-bromo-D-mannose, 6-deoxy-6-chloro-D-mannose, 6-deoxy-6-fluoro-D-galactose, 6-deoxy-6-chloro-D-galactose, 6-deoxy-6-iodo-D-galactose, 6-deoxy-6-bromo-D-galactose, halogenated C-6 sugars gluconolactones, glucuronic acid, glucopyranoside, and their phosphate derivatives, glucuronides with halogenated glycosides at the C-1 position, C-2 substituted D-hexoses, 2-deoxy-2-halogeno-D-hexoses, 2-deoxy-2-fluoro-D-glucose, 2-chloro-2-deoxy-D-glucose, 2-bromo-D-glucose, 2-iodo-D-glucose, 2-deoxy-2,2-difluoro-D-arabino-hexose, 2-deoxy-2-fluoro-D-mannose, 2-deoxy-D-arabino-hexose, 2-Deoxy-2-fluoro-D-galactose, 1,6-anhydro-2-deoxy-2-fluoro-beta-D-glucopyranose, 1-6-anhydrosugar, 2-amino-2-deoxy-D-glucose, glucose amine, 2-amino-2-deoxy D-galactose, galactosamine, 2-amino-2-deoxy-D-mannose, mannosamine, 2-deoxy-2-fluoro-D-mannose, 2-deoxy-2-fluoro-D-galactose, 2-deoxy-D-arabino-hexose, 2-deoxy-2,2-difluoro-D-arabino-hexose, 2-deoxy-2-fluoro-D-glucose 1-Phosphate, 2-deoxy-2-fluoro-D-glucose 6-P, 2-deoxy-2-fluoro-D-glucose 1,6 biphosphate, 2-deoxy-2-fluoro-D-mannose 1-P, 2-deoxy-2-fluoro-D-mannose 6-P, 2-deoxy-2-fluoro-D-mannose 1,6-biphosphate, nucleotide diphosphate, uridine di-P, 1-2 deoxy-2-fluoro-D-glucose, C-2-halogen substituted, and NH3 substituted derivatives of D-Glucose 6-phosphate, 2-deoxy-2-fluoro-2-D-glucose-6-phosphate, 2-chloro-2-deoxy-D-glucose-6-phosphate, 2-deoxy-D-arabino-hexose-6-phosphate, D-glucosamine-6-phosphate, 2-deoxy-2-fluoro-2-D-mannose-6-P, and any known derivatives, C-2 halogenated derivatives of hexose ring pyranoses, mannopyranoses, galactopyranoses, C-2-deoxy-2- fluoropyranoses, and any derivative, C-2 halogenated

sugars derivatives, C-2 fluoro-, bromo-, chloro-, or iodo-sugars derivatives, fluoro, bromo, chloro, or iodo C-2 sugars derivatives, gluconolactones, glucuronic acid, glucopyranoside, and their phosphate derivatives, sugars modified at C-1 or C-5 by replacement of hydroxyl by fluorine or deoxygenation or replacement by a sulfur group, glucosyl fluoride, 1-deoxy-D-
5 glucose, 5-thio-D-glucose, 3-deoxy or 3-fluoro-D-glucose or 4-deoxy or 4-fluoro-D-glucose, 2-fluoro- or 2-iodo-, or 2-thio-, or 2-methoxy- or 3-fluoro-, or 3, 3 difluoro-, 3-iodo-, or 3-carboxylo-, or 3-thio-glyceraldehydes or glycerates, 3-fluoro-2-phosphoglycerate, phosphothioesters or other phosphor modified analogs, mannoheptulose mannoheptose, glucoheptose, N-acetylglucosamine, 6-aminonicotinamide acidosis-inducing agents, 2-
10 deoxy-2-fluoro-D-glucose, citrate and halogenated derivatives of citrate, fructose 2,6-bisphosphate, bromoacetylethanolamine phosphate analogues, N-(2-methoxyethyl)-bromoacetamide, N-(2-ethoxyethyl)-bromoacetamide, N-(3-methoxypropyl)-bromoacetamide), iodoacetate, pentalenolactone, arsenic, 1,1-difluoro-3-phosphate-glycerol, oxamate, 2-fluoro-propionic acid or its salts, 2,2-difluoro-propionic acid, 3-halopropionic acid,
15 or 2-thiomethylacetic acid.

Another embodiment of the invention is a composition as described above, wherein an inhibitor of glycolysis is any of 2FDG, oxamate or iodoacetate.

20 Another embodiment of the invention is a composition as described above, wherein said glycolysis inhibitor, when comprising a halogen atom, comprises a halogen radioisotope in place of a stable halogen atom.

Another embodiment of the invention is a composition as described above, wherein said 25 oxidative phosphorylation inhibitor is an inhibitor of one or more of complex I (NADH coenzyme Q reductase), II (succinate-coenzyme Q reductase), III (coenzyme Q cytochrome C reductase), IV (cytochrome oxydase), and V (F0-F1, ATP synthase).

Another embodiment of the invention is a composition as described above, wherein said 30 oxidative phosphorylation inhibitor is any of rotenone, amytal, 1-methyl-4-phenylpyridinium, paraquat, myxothiazol, antimycin A, ubisemiquinone, cytochrome C, 4,6-diaminotriazine derivatives, cyanide, hydrogen sulfide, azide, formate, phosphine, carbon monoxide, 4'-demethyl-epipodophyllotoxin thenylidene glucoside, tritylthioalanine, carminomycin, piperazinedione, dinitrophenol, dinitrocresol, 2-hydroxy-3-alkyl-1,4-naphthoquinones, 35 apoptolidin aglycone, oligomycin, ossamycin, clofazimine cytovaricin, naphthoquinone

derivatives, dichloroallyl-lawsone, lapachol, rhodamine, rhodamine 123, rhodamine 6G, carbonyl cyanide p-trifluoromethoxyphenylhydrazone, cyhexatin, dichlorodiphenyltrichloroethane (DDT), chlordecone, arsenate, pentachlorophenol, benzonitrile, thiadiazole herbicides, salicylate, cationic amphiphilic drugs, amiodarone, 5 perhexiline, gramicidin, calcimycin, pentachlorobutadienyl-cysteine, trifluorocarbonylcyanide phenylhydrazone, atractyloside, lysophospholipids, n-ethylmaleimide, mersanyl, or p-benzoquinone.

Another embodiment of the invention is a composition as described above, wherein said 10 inhibitor of oxidative phosphorylation is any of rhodamine, rhodamine 6G, rhodamine 123, dinitrophenol, or rotenone.

Another embodiment of the invention is a composition as described above, wherein said oxidative phosphorylation inhibitor, when comprising a halogen atom, comprises a halogen 15 radioisotope in place of a stable halogen atom.

Another embodiment of the invention is a composition as described above, wherein the oxidative phosphorylation inhibitor in an amount such that the concentration of inhibitor delivered to a subject is between 20 and 60 mg/kg.

20 Another embodiment of the invention is a composition as described above, further comprising one or more pyrophosphates.

Another embodiment of the invention is a composition as described above, wherein said 25 pyrophosphate is one or more of sodium pyrophosphate, potassium pyrophosphate, calcium pyrophosphate.

Another embodiment of the invention is a composition as described above, wherein said 30 pyrophosphate is administered simultaneous, separate or sequentially in respect of the inhibitors.

Another embodiment of the invention is a composition as described above, further comprising one or more imaging agents.

Another embodiment of the invention is a composition as described above, wherein said imaging agent is any of poly(ortho)ester, magnesium alloy powder, metallic powder, tantalum powder, biocompatible metal powder, iridium powder, or micro-bubbles.

- 5 Another embodiment of the invention is a composition as described above, wherein said slow release agent is any of poly(glycolic) acid, poly(lactic acid) or in general glycolic- and lactic acid based polymers, copolymers, poly caprolactones and in general, poly hydroxyl alkanoate,s poly(hydroxy alcanoic acids), Poly (ethylene glycol), poly vinyl alcohol, poly (orthoesters), poly (anhydrides), poly (carbonates), poly amides, poly imides, poly imines,
- 10 poly (imino carbonates), poly (ethylene imines), polydioxanes, poly oxyethylene (poly ethylene oxide), poly (phosphazenes), poly sulphones, lipids, poly acrylic acids, poly methylmethacrylate, poly acryl amides, poly acrylo nitriles (Poly cyano acrylates), poly HEMA, poly urethanes, poly olefins, poly styrene, poly terephthalates, poly ethylenes, poly propylenes, poly ether ketones, poly vinylchlorides, poly fluorides, silicones, poly silicates
- 15 (bioactive glass), siloxanes (Poly dimethyl siloxanes), hydroxyapatites,poly aminoacids (natural and non natural), poly β-aminoesters, albumines, alginates, cellulose / cellulose acetates, chitin / chitosan, collagene, fibrine / fibrinogen, gelatine, lignine, proteine based polymers, Poly (lysine), poly (glutamate), poly (malonates), poly (hyaluronic acids), Poly nucleic acids, poly saccharides, poly (hydroxyalkanoates), poly isoprenoids, starch based
- 20 polymers, copolymers thereof, linear, branched, hyperbranched, dendrimers, crosslinked, functionalised derivatives thereof, hydrogels based on activated polyethyleneglycols combined with alkaline hydrolyzed animal or vegetal proteins.

- 25 Another embodiment of the invention is a composition as described above, wherein said composition further comprises magnesium alloys.

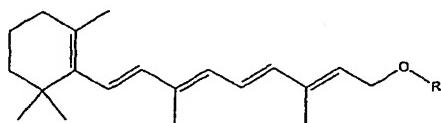
- Another embodiment of the invention is a composition as described above, wherein at least one of said inhibitors is coupled to solubilising agent.
- 30 Another embodiment of the invention is a composition as described above, wherein at least one said solubilising agent is cholesterol or derivative thereof.

- Another embodiment of the invention is a composition as described above, wherein said cholesterol derivatives are any of cholestryl-3-betahydroxybutyrate, cholestryl-halogenated butyrate, cholestryl-halogenated acetate, cholestryl-halogenated aceto-acetate,
- 35

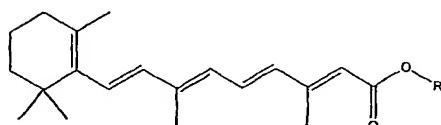
cholesteryl-halogenated acetamide, cholesteryl-halogenated crotonate, cholesteryl-halogenated acetone, cholesteryl-halogenated citrate, or cholesteryl-halogenated oleate.

- Another embodiment of the invention is a composition as described above, wherein at least
5 one said solubilising agent is vitamin A or derivative thereof.

Another embodiment of the invention is a composition as described above, wherein
derivative of vitamin A is formula (IV) or (V) below



(IV)



(V)

10

wherein R is selected from the group consisting of betahydroxybutyrate, halogenated butyrate, halogenated acetate, halogenated aceto-acetate, halogenated acetamide, halogenated crotonate, halogenated acetone, halogenated citrate, and halogenated oleate.

- 15 Another embodiment of the invention is a composition as described above, wherein at least one of said inhibitors is present in micro-capsule and/or nano-capsule.

- Another embodiment of the invention is a composition as described above, wherein nano-capsule is any of copolymer poly(ethylene oxide) with poly(L-Lactic acid) or with poly(beta-20 benzyl-L-aspartate), copolymer with poly(lactide-co-glycolide)-[(propylene oxide)-poly(ethylene oxide)], polyphosphazene derivatives, poly(ethylene glycol) coated nanospheres, poly(isobutylcyanoacrylate) nanocapsules, poly(gamma-benzyl-L-glutamate)/(poly(ethylene oxide), chitosan-poly(ethylene oxide) nanoparticles, nanoparticles where said inhibitor is prepared using o-carboxymethylate chitosan as wall forming material,
25 or solid lipid nanospheres (SLN).

- Another embodiment of the invention is a composition as described above, wherein micro-capsule is any of multiporous beads of chitosan, coated alginate microspheres, N-(aminoalkyl) chitosan microspheres, chitosan/calcium alginate beads, poly(adipic anhydride) microspheres, gellan-gum beads, poly(D, L-lactide-co-glycolide) microspheres, alginate-poly-L-lysine microcapsules, crosslinked chitosan microspheres, chitosan/gelatin microspheres, crosslinked chitosan network beads with spacer groups, 1,5-diozepan-2-one microspheres, D,L-dilactide microspheres, triglyceride lipospheres, polyelectrolyte complexes of sodium alginate chitosan, polypeptide microcapsules, or albumin microspheres.
- 10 Another embodiment of the invention is a composition as described above, wherein said composition is part of a solid wall composition.
- Another embodiment of the invention is a composition as described above, wherein said 15 solid wall composition is a capsule of suitable size and shape for administration using a needle, said capsule filled with composition.
- Another embodiment of the invention is a composition as described above, wherein a wall of 20 said capsule comprises gelatin.
- Another embodiment of the invention is a composition as described above, wherein said solid wall composition is a solid state bioabsorbable structure of suitable size and shape for administration using a needle, said structure impregnated with composition.
- 25 Another embodiment of the invention is a composition as described above, wherein said solid state bioabsorbable structure is seed-shaped, rod-shaped, or tube-shaped.
- Another embodiment of the invention is a composition as described above, wherein at least 30 one said inhibitor is a derivative of the inhibitor which is a stereoisomer, tautomer, racemate, prodrug, metabolite, pharmaceutically acceptable salt, base, ester, structurally related compounds or solvate.
- Another embodiment of the invention is a composition as described above, for the treatment 35 of cellular proliferation, wherein said composition is administered into the proliferating cell mass.

Another embodiment of the invention is a use of a composition as described above for the manufacture of a medicament for the treatment of cellular proliferation, wherein said composition is administered into the proliferating cell mass.

5

Another embodiment of the invention is a use of a composition as described above for the manufacture of a medicament for sensitising a cellular proliferation to treatment by radiotherapy, wherein said composition is administered into the proliferating cell mass prior to radiotherapy.

10

Another embodiment of the invention is a use of a composition as described above for the manufacture of a medicament for sensitising a cellular proliferation to treatment by chemotherapy, wherein said composition is administered into the proliferating cell mass prior to chemotherapy.

15

Another embodiment of the invention is a use or composition as described above, wherein said TCA cycle, glycolysis and oxidative phosphorylation inhibitors are administered separately, simultaneously or sequentially.

20

Another embodiment of the invention is a use or composition as described above, further combined with radiotherapy.

Another embodiment of the invention is a use or composition as described above, further combined with chemotherapy.

25

Another embodiment of the invention is a use or composition as described above, wherein said composition is administered by injection into a mass of proliferating cells.

30

Another embodiment of the invention is a use or composition as described above, wherein said composition is administered by infusion into a mass of proliferating cells.

Another embodiment of the invention is a use or composition as described above, wherein said composition is administered by high-pressure injection into a mass of proliferating cells.

Another embodiment of the invention is a use or composition as described above, wherein said composition is administered in the resection cavity or scar of a proliferating mass.

Another embodiment of the invention is a kit comprising a composition comprising one or 5 more inhibitors of the TCA cycle, one or more inhibitors of glycolysis and one or more inhibitors of oxidative phosphorylation.

Another embodiment of the invention is a kit as described above, wherein said composition is a composition as defined above.

10 Another embodiment of the invention is a kit as described above, further comprising a syringe.

Another embodiment of the invention is a hydrogel comprising a) composition as defined 15 above, and
b) an activated polyethyleneglycol (PEG) combined with any of alkaline hydrolyzed soya solutions, animal or vegetal proteins, bovine serum albumin, soya globulin, casein, pea albumin, starch albumine, or ovalbumin.

20 Another embodiment of the invention is a hydrogel as defined above wherein a TCA inhibitor of the composition is present at a concentration of less than or equal to 0.1 mg per square cm of hydrogel and/or a glycolysis inhibitor of the composition is present at a concentration of less than or equal to 10 mg per square cm of hydrogel and/or an oxidative phosphorylation inhibitor of the composition is present at a concentration of less than or equal to 1 mg per 25 square cm of hydrogel.

Another embodiment of the invention is a use of a hydrogel as defined above for treatment of superficial cell proliferation, such as basal carcinoma or a squamous cell carcinoma by application of the hydrogel to the surface of said proliferations.

30 **DETAILED DESCRIPTION OF THE INVENTION**

The present invention relates to the use of substances that block the citric acid cycle (TCA cycle), glycolysis and oxidative phosphorylation, for the treatment of the proliferation of cells.

Proliferative or proliferating cells, whether benign or malignant, are cells such cancer cells, vascular restenosis cells, hypertrophic scar cells, cheloid cells, inflammatory cells, benign tumor cells or any rapidly proliferating cell. Such cells are found in, for example, tumours, hyperproliferative scars, cheloid scars, myoma or fibroma benign tumors and restenotic processes or tumors at the level of a duct. Generally such cells rapidly proliferate (hyperproliferative). A collection of such cells form a cell mass.

Magnetic resonance spectroscopy studies performed by the inventors have shown the TCA cycle is more active or highly active in a majority of hyperproliferative cells, compared with adjacent cells not undergoing hyperproliferation. This property enables one or more TCA cycle inhibitors to be employed proximal to the site of cell proliferation, and to be rapidly taken up by said proliferating cells in doses higher than by non-proliferating cells. Consequently high doses of inhibitor or extremely potent inhibitors may be applied locally, resulting in cell death of hyperproliferative cells, and reduced or no cell death of non-proliferating cells. The differential in TCA metabolism can allow for a reduction in the amount of active substance necessary compared with conventional chemotherapy.

The inventors have also found that even further cell death is achieved when glycolysis and oxidative phosphorylation are additionally inhibited.

One embodiment of the present invention is a composition comprising one or more inhibitors of the TCA cycle, one or more inhibitors of glycolysis and one or more inhibitors of oxidative phosphorylation.

Another embodiment of the present invention is a composition comprising one or more inhibitors of the TCA cycle, one or more inhibitors of glycolysis and one or more inhibitors of oxidative phosphorylation for treating hyperproliferative cells administered to or in the region of the proliferation, preferably into the proliferating cell mass.

Another embodiment of the present invention is a use of a composition comprising one or more inhibitors of the TCA cycle, one or more inhibitors of glycolysis and one or more inhibitors of oxidative phosphorylation for the preparation of a medicament for treating hyperproliferative cells, wherein the composition is administered to or in the region of the proliferation, preferably into the proliferating cell mass.

Another embodiment of the present invention is a use of a composition comprising one or more inhibitors of the TCA cycle, one or more inhibitors of glycolysis, one or more inhibitors of oxidative phosphorylation and one or more slow release agents for the preparation of a medicament for treating hyperproliferative cells, wherein the composition is administered to
5 or in the region of the proliferation, preferably into the proliferating cell mass.

The present invention also relates to a method for sensitising proliferating cells present in a
10 cavity of a subject to treatment by radiotherapy and/or chemotherapy, comprising administering into the proliferating cell mass a composition comprising one or more inhibitors of the TCA cycle, one or more inhibitors of glycolysis and one or more inhibitors of oxidative phosphorylation prior to said radiotherapy and/or chemotherapy.

15 One embodiment of the present invention is a method for the treatment of cellular proliferation comprising administering to a patient a composition comprising one or more inhibitors of the TCA cycle, one or more inhibitors of glycolysis and one or more inhibitors of oxidative phosphorylation for treating hyperproliferative cells, said administration to or in the region of the proliferation, preferably into the proliferating cell mass.
20

The composition can be a pharmaceutical composition. Where a particular use of a composition of the present invention is described, said use may be understood as a method. In the preferred mode of the invention, an inhibitor of oxidative phosphorylation is rhodamine (i.e. rhodamine, rhodamine 6G, rhodamine 123). In another preferred mode of the invention
25 an inhibitor of the TCA cycle is fluoroacetate. In another preferred mode of the invention an inhibitor of glycolysis is 2FDG (2-deoxy-2-fluoro-D-glucose). In another preferred mode of the invention a slow release agent is polyorthoester. The present invention may be applied using any inhibitors of these pathways as indicated below. The composition may be administered to or in the region of the proliferation. Thus, composition is therefore, administered locally to
30 the site of proliferation, and not systemically. Preferably it is administered into the proliferating cell mass.

TCA cycle inhibitors

A TCA cycle inhibitor of the invention is any inhibitor of one or more enzymes of the TCA cycle. The TCA cycle enzymes are known in the art and include pyruvate dehydrogenase, citrate synthase, aconitase, isocitrate lyase, alpha-ketoglutarate dehydrogenase complex, succinyl CoA synthetase, succinate dehydrogenase, fumarase, malate synthase, 5 glutaminase, and pyruvate dehydrogenase complex. It is an aspect of the invention that an inhibitor of aerobic ATP synthesis is an inhibitor of an enzyme associated with the TCA cycle. Inhibitors of the TCA cycle are any known in the art.

The availability of reduced and oxidized forms of nicotinamide adenine dinucleotide (NAD⁺ and NADH) is important for the TCA and depletors of NAD⁺ and NADH would be inhibitors 10 of the TCA cycle. Depletors of NAD⁺ and/or NADH include Hypoglycin A and its metabolite methylenecyclopropylacetic acid, ketone bodies (D(-)-3-hydroxybutyrate), alloxan, PNU and any other substance known in the art.

15 Inhibitors of pyruvate dehydrogenase are any known in the art and may include, but are not limited to any of arsenite, dichlorovinyl-cysteine, p-benzoquinone, thiaminase.

Inhibitors of citrate synthetase are any known in the art and may include, but are not limited to any of the following:

20 Fluoroacetate (and its derivative fluoroacetyl-CoA), any halogenated acetyl-CoA, fluoroacetamide, fluorocrotonate, halogenated ketone bodies (for instance, chloroacetoacetate, fluoroacetoacetate, fluorohydroxybutyrate, chlorohydroxybutyrate, bromohydroxybutyrate), halogenated acetone, halogenated acetic acid (for example chloracetic acid), halogenated oleate (an analogue of ketone bodies) and any known in the 25 art.

Inhibitors of aconitase are any known in the art and may include, but are not limited to any of the following:

Fluorocitrate, fluorocitrate 2R, 3R, and any other halogenated citrate (bromocitrate, 30 chlorocitrate).

Inhibitors of isocitrate dehydrogenase are any known in the art and may include, but are not limited to any of the following:

DCVC (dichlorovinyl-cysteine)

Inhibitors of succinate dehydrogenase are any known in the art and may include, but are not limited to malonate, DCVC, Pentachlorobutadienyl-cysteine (or PCBD-cys), 2-bromohydroquinone, 3-nitropropionic acid, cis-crotonalide fungicides.

- 5 Inhibitors of succinyl CoA synthetase, alpha ketoglutarate dehydrogenase complex, fumarate hydratase (fumarase), or malate dehydrogenase are any known in the art.

Inhibitors of glutaminase are any known in the art and may include, but are not limited to 6-diazo-5-oxo-L-norleucine (DON).

- 10 Inhibitors of glutamate dehydrogenase are any known in the art.

Other inhibitors of the TCA cycle include glu-hydroxyoxamate, p-chloromercuriphenylsulphonic acid (impermeant thiol agent), L-glutamate gamma-15 hydroxamate, p-chloromercuriphenylsulphonic acid, acivicin (alpha-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid), halogenated glutamine and glutamate.

- Several other compounds may block the production of ATP at the level of the TCA cycle after compound transformation. Indeed, most amino-acids may be degraded to enter the TCA 20 cycle at various places. Therefore, most of aminoacids used in an halogenated formulation will be able to block the TCA cycle by being degraded to one of the TCA products (halogenated glutamate, glutamine, histidine, proline, arginine, valine, methionine, threonine, isoleucine, aspartate, tyrosine, phenylalanine, asparagine, aspartate, alanine, glycine, cysteine, serine, threonine). Some of the amino-acids will be transformed into ketone bodies. 25 These amino-acids (leucine, lysine, phenylalanine, tyrosine) in an halogenated presentation will interact at the same sites where halogenated ketone bodies interact as described previously. Finally, some amino-acids in an halogenated formulation (tryptophan, leucine, isoleucine) will be directly tranformed into acetyl-CoA and will block the TCA at the level of citrate synthase - aconitase.

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- According to another embodiment of the invention, a TCA cycle inhibitor is any of arsenite, hypoglycin A, methylenecyclopropylacetic acid, alloxan, PNU, p-benzoquinone, fluoroacetate, halogenated acetates (iodo-, bromo-, chloro-acetate), halogenated acetyl-CoA 35 (fluoroacetyl-CoA, bromoacetyl-CoA, chloroacetyl-CoA, iodoacetyl-CoA), halogenated

- crotonate (fluoro-, iodo-, bromo-, chloro-crotonate); halogenated ketone bodies, (chloro-, fluoro-, bromo-, iodoaceto-acetate, fluoro-, chloro-, bromo-, iodo-butyrate, fluoro-, chloro-, bromo-, iodo-acetone), halogenated oleate (iodo, bromo, chloro, fluoro-oleate), halogenated citrate, halogenated citrate 2R, 3R isomer (fluoro-, bromo-, chloro-, iodo-citrate),
- 5 dichlorovinyl-cysteine, halogenated aminoacids, malonate, pentachlorobutadienyl-cysteine, 2-bromohydroquinone, 3-nitropropionic acid, cis-crotonalide fungicides, glu-hydroxyoxamate, p-chloromercuriphenylsulphonic acid, L-glutamate gamma-hydroxamate, p-chloromercuriphenylsulphonic acid, acivicin (alpha-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid, halogenated glutamine (fluoro, iodo, chloro, bromo-glutamine), or
- 10 halogenated glutamate (fluoro, iodo, chloro, bromo-glutamate).

Where more than one inhibitor of the TCA is present in a composition, preferably, one inhibitor is directed towards the upper half of the TCA cycle, which is characterised by providing no redox products such as NADH, HANPH, or FADH₂ (e.g. enzymes pyruvate dehydrogenase, citrate synthase, aconitase) and another inhibitor is directed towards the lower half of the TCA cycle, which is characterised by providing redox products such as NADH, HANPH, or FADH₂ (e.g. enzymes isocitrate lyase, alpha-ketoglutarate dehydrogenase complex, succinyl CoA synthetase, succinate dehydrogenase, malate synthase, glutaminase). Examples of a combination of inhibitor includes fluoroacetate and

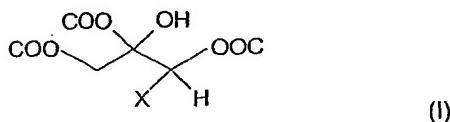
15 malonate.

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Fluorocitrate and derivatives

According to a preferred embodiment of the invention, a TCA cycle inhibitor of the invention has a formula (I):

25



- 30 where X may be halide, a sulfonate, a carboxylate, an alkoxide, an amine oxide or a OH. The halide may be selected from the group consisting of: fluoride, bromide, chloride, and iodide. The sulfonate may be selected from the group consisting of: triflate, mesylate and tosylate.

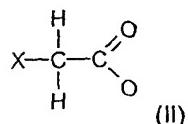
The carboxylate may be selected from the group consisting of: methoxylate and ethyloxylate. The alkoxide may be selected from the group consisting of: methoxide and ethoxide. The amine oxide is dimethylamine oxide. According to one aspect of the invention, the stereochemistry is 2R, 3R.

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Fluoroacetate and derivatives

TCA cycle inhibitors also includes substances which are converted into inhibitors of the TCA cycle such as, for example fluoroacetate and derivatives. According to a preferred embodiment of the invention, a TCA cycle inhibitor of the invention has a formula (II):

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where X may be halide, a sulfonate, a carboxylate, an alkoxide, or an amine oxide, a OH. The halide may be selected from the group consisting of: fluoride, bromide, chloride, and iodide. The sulfonate may be selected from the group consisting of: triflate, mesylate and tosylate. The carboxylate may be selected from the group consisting of: methoxylate and ethyloxylate. The alkoxide may be selected from the group consisting of: methoxide and ethoxide. The amine oxide may be dimethylamine oxide.

20 Preferably, an inhibitor of the TCA cycle is any of fluoroacetate, fluorocitrate, arsenite, acetoacetate, and betahydroxy butyrate.

Halogenated inhibitors which are radioisotopes

According to an aspect of the present invention, at least one halogen atom where present in 25 an inhibitor of the TCA cycle is substituted for the corresponding halogen atom radioisotope, to form a radio-isotope-halogen TCA cycle inhibitor (RIH-TCA cycle inhibitor). The radioisotopes of halides may be for example ¹⁸F, ⁷⁹Br, ⁸¹Br, ³⁶Cl, ¹²⁵I, ¹²⁹I, ¹³¹I, which all emit ionising radiation. For example, the stable fluorine atom of fluoroacetate may be substituted for ¹⁸F to form ¹⁸F-fluoroacetate. Similarly ¹²⁹I-iodoacetate may be employed as a TCA cycle 30 inhibitor. The use of an RIH-TCA cycle inhibitor allows effective brachytherapy, simultaneously with pathway inhibition as described above. Furthermore, where used in combination therapy the RIH-TCA cycle inhibitor can be administered sequentially after an

oxidative phosphorylation and/or glycolysis inhibitor; the proliferating cells, therefore, are affected both in terms of energy production by the inhibitors and by the ionising radiation of the RIH-TCA cycle inhibitor. The dose of the RIH-TCA cycle inhibitor can be adjusted so that the cytotoxic effect is due to the ionising radiation rather than pathway inhibition, or *vice versa.*

Simultaneous inhibition of other pathways

According to an aspect of the invention a TCA cycle inhibitor is capable of inhibiting at least 3 cellular mechanisms of proliferating cells simultaneously. This may be achieved by blocking, for example, aconitase from the TCA cycle. The inventors have realised that the use of an aconitase inhibitor such as, for example, fluorocitrate (or fluoroacetate which is later converted into fluorocitrate) can inhibit other important pathways such as fatty acid synthesis at the level of ATP-citrate lyase and calcium intracellular signalling through derivatives accumulation.

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Glycolysis inhibitors

According to one embodiment of the invention, an inhibitor of glycolysis inhibits at least one enzyme from the group consisting of hexokinase, glucokinase, phosphoglucose isomerase, phosphofructokinase, aldolase, triose phosphate isomerase, glyceraldehyde 3-phosphate dehydrogenase, phosphoglycerate kinase, phosphoglyceromutase, enolase, pyruvate kinase, and lactate dehydrogenase.

According to another embodiment of the invention, an inhibitor of glycolysis is a hexose sugar modified by removal of the hydroxyl group or by the substitution of the hydroxyl group with halogen atom or thiol at:

- C6 for inhibiting hexokinase,
- C1 or C2 or C5 for inhibiting phosphoglucoisomerase
- C3 and/or C4 for blocking aldolase, and/or
- C2 or C3 for blocking glyceraldehyde 3P deshydrogenase, phosphoglycerate kinase, phosphoglycerate mutase, and enolase.

According to another embodiment of the invention, an inhibitor of glycolysis is any of 6-deoxy-6-fluoro-D-glucose, 6-deoxy-6-bromo-D-glucose, 6-deoxy-6-chloro-D-glucose, 6-O-methyl-D-glucose, 6-thio-D-glucose, 6-deoxy-D-glucose, C-6 modified or blocked derivatives of other hexose ring pyranoses, mannopyranoses, galactopyranoses, 6-deoxy-6-fluoro-D-

glucose, 6-deoxy-6-bromo-D-mannose, 6-deoxy-6-chloro-D-mannose, 6-deoxy-6-fluoro-D-galactose, 6-deoxy-6-chloro-D-galactose, 6-deoxy-6-iodo-D-galactose, 6-deoxy-6-bromo-D-galactose, halogenated C-6 sugars gluconolactones, glucuronic acid, glucopyranoside, and their phosphate derivatives, glucoronides with halogenated glycosides at the C-1 position, C-2 substituted D-hexoses, 2-deoxy-2-halogeno-D-hexoses, 2-deoxy-2-fluoro-D-glucose (2FDG), 2-chloro-2-deoxy-D-glucose, 2-bromo-D-glucose, 2-iodo-D-glucose, 2-deoxy-2,2-difluoro-D-arabino-hexose, 2-deoxy-2-fluoro-D-mannose, 2-deoxy-D-arabino-hexose, 2-Deoxy-2-fluoro-D-galactose, 1,6-anhydro-2-deoxy-2-fluoro-beta-D-glucopyranose, 1-6-anhydrosugar, 2-amino-2-deoxy-D-glucose, glucose amine, 2-amino-2-deoxy D-galactose, galactosamine, 2-amino-2-deoxy-D-mannose, mannosamine, 2-deoxy-2-fluoro-D-mannose, 2-deoxy-2-fluoro-D-galactose, 2-deoxy-D-arabino-hexose, 2-deoxy-2,2-difluoro-D-arabino-hexose, 2-deoxy-2-fluoro-D-glucose 1-Phosphate, 2-deoxy-2-fluoro-D-glucose 6-P, 2-deoxy-2-fluoro-D-glucose 1,6 biphosphate, 2-deoxy-2-fluoro-D-mannose 1-P, 2-deoxy-2-fluoro-D-mannose 6-P, 2-deoxy-2-fluoro-D-mannose 1,6-biphosphate, nucleotide diphosphate, uridine di-P, 1-2 deoxy-2-fluoro-D-glucose, C-2-halogen substituted, and NH3 substituted derivatives of D-Glucose 6-phosphate, 2-deoxy-2-fluoro-2-D-glucose-6-phosphate, 2-chloro-2-deoxy-D-glucose-6-phosphate, 2-deoxy-D-arabino-hexose-6-phosphate, D-glucosamine-6-phosphate, 2-deoxy-2-fluoro-2-D-mannose-6-P, and any known derivatives, C-2 halogenated derivatives of hexose ring pyranoses, mannopyranoses, galactopyranoses, C-2-deoxy-2-fluoropyranoses, and any derivative, C-2 halogenated sugars derivatives, C-2 fluoro-, bromo-, chloro-, or iodo-sugars derivatives, fluoro, bromo, chloro, or iodo C-2 sugars derivatives, gluconolactones, glucuronic acid, glucopyranoside, and their phosphate derivatives, sugars modified at C-1 or C-5 by replacement of hydroxyl by fluorine or deoxygenation or replacement by a sulfur group, glucosyl fluoride, 1-deoxy-D-glucose, 5-thio-D-glucose, 3-deoxy or 3-fluoro-D-glucose or 4-deoxy or 4-fluoro-D-glucose, 2-fluoro- or 2-ido-, or 2-thio-, or 2-methoxy- or 3-fluoro-, or 3, 3 difluoro-, 3-iodo-, or 3-carboxylo-, or 3-thio-glyceraldehydes or glycerates, 3-fluoro-2-phosphoglycerate, phosphothioesters or other phosphor modified analogs, mannoheptulose mannoheptose, glucoheptose, N-acetylglucosamine, 6-aminonicotinamide acidosis-inducing agents, 2-deoxy-2-fluoro-D-glucose, citrate and halogenated derivatives of citrate, fructose 2,6-bisphosphate, bromoacetylethanolamine phosphate analogues, N-(2-methoxyethyl)-bromoacetamide, N-(2-ethoxyethyl)-bromoacetamide, N-(3-methoxypropyl)-bromoacetamide), iodoacetate, pentalenolactone, arsenic, 1,1-difluoro-3-phosphate-glycerol, oxamate, 2-fluoro-propionic acid or its salts, 2,2-difluoro-propionic acid, 3-halopropionic acid, and 2-thiomethylacetic acid.

Preferably, an inhibitor of glycolysis is any of 2FDG, oxamate or iodoacetate.

Glycolysis is the main pathway for anaerobic ATP synthesis. Tumours switch to anaerobic ATP synthesis by metabolising the well-distributed glucose among others in order 5 to provide nucleotides through the PPP pathway. It is known that proliferating masses which are partly under anaerobic type respiration are more resistant to radiation or chemotherapy. Therefore, by locally inhibiting the glycolysis pathway, anaerobic respiration which is the principal energy pathway of poorly oxygenated cells is inhibited, leading to increased cell 10 death of hypoxic proliferating cells. The proliferation of non-hypoxic cells is slowed as well owing to the shutdown of this primary energy pathway.

Halogenated inhibitors which are radioisotopes

According to an aspect of the present invention, at least one halogen atom where present in 15 an inhibitor of glycolysis is substituted for the corresponding halogen atom radioisotope, to form a radio-isotope-halogen glycolysis inhibitor (RIH-glycolysis inhibitor). The radioisotopes of halides may be for example ^{18}F , ^{79}Br , ^{81}Br , ^{36}Cl , ^{125}I , ^{129}I , ^{131}I , which all emit ionising radiation. For example, the stable fluorine atom of 2FDG may be substituted for ^{18}F to form ^{18}F -2FDG. Similarly -6-deoxy-6- ^{129}I -D-galactose may be employed as a glycolysis inhibitor. 20 The use of an RIH-glycolysis inhibitor allows effective brachytherapy, simultaneously with pathway inhibition as described above. Furthermore, where used in combination therapy the RIH-glycolysis inhibitor can be administered sequentially after an oxidative phosphorylation and/or glycolysis inhibitor; the proliferating cells, therefore, are affected both in terms of 25 energy production by the inhibitors and by the ionising radiation of the RIH-glycolysis inhibitor. The dose of the RIH-glycolysis inhibitor can be adjusted so that the cytotoxic effect is due to the ionising radiation rather than pathway inhibition, or vice versa.

Oxidative phosphorylation inhibitors

According to one embodiment of the invention, an inhibitor of glycolysis inhibits at least one 30 enzyme from the group consisting of enzyme complex I (NADH coenzyme Q reductase), II (succinate-coenzyme Q reductase), III (coenzyme Q cytochrome C reductase), IV (cytochrome oxydase), and V (F0-F1, ATP synthase).

Inhibitors of enzyme complex I are any known in the art and may include, but are not limited 35 to any of the following: tritylthioalanine, carminomycin, and piperazinedione, rotenone,

amytal, 1-methyl-4-phenylpyridinium (MPP⁺), paraquat, methylene blue, Ferricyanide (the latter 2 are electron acceptors).

Inhibitors of enzyme complex II are any known in the art.

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Inhibitors of coenzyme Q are any known in the art.

Inhibitors of enzyme complex III are any known in the art and may include, but are not limited to myxothiazol, antimycin A, ubisemiquinone, cytochrome C, 4,6-diaminotriazine derivatives, 10 metothrexate or electron acceptors such as phenazine methosulfate and 2,6-Dichlorophenol-indophenol.

Inhibitors of enzyme complex IV are any known in the art and may include, but are not limited to cyanide, hydrogen sulfide, azide, formate, phosphine, carbon monoxide and 15 electron acceptor ferricyanide.

Inhibitors of enzyme complex V are any known in the art and may include, but are not limited to VM-26 (4'-demethyl-epipodophyllotoxin thenylidene glucoside), tritylthioalanine, carminomycin, piperazinedione, dinitrophenol, dinitrocresol, 2-hydroxy-3-alkyl-1,4- 20 naphtoquinones, apoptolidin aglycone, oligomycin, ossamycin, cytovaricin, naphtoquinone derivatives (e.g. dichloroallyl-lawsone and lapachol), rhodamine, rhodamine 123, rhodamine 6G, carbonyl cyanide p-trifluoromethoxyphenylhydrazone, rothenone, safranine O, cyhexatin, DDT, chlordecone, arsenate, pentachlorophenol, benzonitrile, thiadiazole herbicides, salicylate, cationic amphiphilic drugs (amiodarone, perhexiline), gramicidin, calcimycin, 25 pentachlorobutadienyl-cysteine (PCBD-cys), trifluorocarbonylcyanide phenylhydrazone (FCCP).

Other inhibitor of oxidative phosphorylation may include atactyloside, DDT, free fatty acids, lysophospholipids, n-ethylmaleimide, mersanyl, p-benzoquinone.

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Preferably, an inhibitor of oxidative phosphorylation is any of rhodamine (*i.e.* rhodamine, rhodamine 6G, rhodamine 123), dinitrophenol, or rotenone.

Where rhodamine, rhodamine 123, or rhodamine 6G are present in the composition, they 35 may be used as inhibitors of oxidative phosphorylation, and not as a dye for homogeneous

coating control, or for photodynamic therapy, for instance. Therefore, where rhodamine compounds are used, the treatment according to the invention is not in combination with rhodamine-based imaging or light-based treatment.

5 *Halogenated inhibitors which are radioisotopes*

According to an aspect of the present invention, at least one halogen atom where present in an inhibitor of oxidative phosphorylation is substituted for the corresponding halogen atom radioisotope, to form a radio-isotope-halogen oxidative phosphorylation cycle inhibitor (RIH-oxidative phosphorylation inhibitor). The radioisotopes of halides may be for example ¹⁸F, ⁷⁹Br, ⁸¹Br, ³⁶Cl, ¹²⁵I, ¹²⁹I, ¹³¹I, which all emit ionising radiation. For example, a stable fluorine atom of pentachlorophenol may be substituted for ¹⁸F to form ¹⁸F-pentachlorophenol. The use of an RIH-oxidative phosphorylation inhibitor allows effective brachytherapy, simultaneously with pathway inhibition as described above. Furthermore, where used in combination therapy, the RIH-oxidative phosphorylation inhibitor can be administered sequentially after a TCA cycle and/or glycolysis inhibitor; the proliferating cells, therefore, are affected both in terms of energy production by the inhibitors and by the ionising radiation of the RIH- oxidative phosphorylation inhibitor. The dose of the RIH-oxidative phosphorylation inhibitor can be adjusted so that the cytotoxic effect is due to the ionising radiation rather than pathway inhibition.

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Simultaneous, sequential and separate

According to one aspect of the invention, the components of the composition – inhibitors of TCA cycle, glycolysis and oxidative phosphorylation - may be administered simultaneously, 25 separately or sequentially in respect of each other.

Another aspect of the invention is a composition comprising at least one TCA cycle inhibitor, at least one glycolysis inhibitor and at least one inhibitor of oxidative phosphorylation, for simultaneous, separate or sequential administration to a subject.

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One aspect of the invention is a method for treating cellular proliferation comprising administering to an individual an effective amount of at least one TCA cycle inhibitor of the invention, at least one glycolysis inhibitor and at least one inhibitor of oxidative phosphorylation, simultaneously, separately or sequentially.

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By simultaneous administration means two or more of the TCA cycle inhibitor, glycolysis inhibitor and oxidative phosphorylation inhibitor are administered to a subject at the same time. For example, as a mixture or a composition comprising said components. An example is as a solution comprising the components of interest.

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By separate administration means that two or more of the inhibitors of the TCA cycle inhibitor, glycolysis inhibitor and oxidative phosphorylation inhibitor are administered to a subject at the same time or substantially the same time. The components may be present in a kit as separate, unmixed preparations. For example, the TCA cycle inhibitor, glycolysis inhibitor and oxidative phosphorylation inhibitor may be present in the kit as individual vials. 10 The inhibitors may be administered to the subject by separate injections at the same time, or injection directly following the other. In another example, the TCA cycle inhibitor and glycolysis inhibitor may be administered together in a solution, and the third component, oxidative phosphorylation inhibitor administered separately.

15

For example, in a prostate cancer, 12 punctures may be performed in the peripheral area of the prostate. One out of each second puncture is made with a composition comprising a slow release formulation of fluoroacetate and 2FDG. The other punctures are made with a composition comprising a slow release formulation of rhodamine 123. Injection sessions may 20 be repeated several times, depending on PSA levels.

25

In another example, a slow release formulation of a TCA cycle inhibitor may be injected inside a proliferative process such as a tumor, a preparation of glycolysis inhibitors may be delivered through an infusion at the same time, followed by a subsequent infusion of oxidative phosphorylation inhibitors. The infusion may be intravenous, intra-arterial or intra-tumoural, under high pressure.

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The therapeutic substances may also be mixed with a slow release agent. It is also possible to administer one formulation first, and the other formulations later on, if PSA levels do not come down completely after the first therapy (for a prostate cancer treatment).

35

By sequential administration means that two or more of the inhibitors of the group TCA cycle inhibitor, glycolysis inhibitor and oxidative phosphorylation inhibitor are administered to a subject sequentially. The TCA cycle inhibitor, glycolysis inhibitor and oxidative phosphorylation inhibitor may be present in a kit as separate, unmixed preparations.

- Alternatively, any two of the TCA cycle inhibitor, glycolysis inhibitor and oxidative phosphorylation inhibitor may be present in a kit together as mixed preparations, the other inhibitor being present in a separate formulation. There is a time interval between doses. For example, one component might be administered up to 336, 312, 288, 264, 240, 216, 192,
- 5 168, 144, 120, 96, 72, 48, 24, 20, 16, 12, 8, 4, 2, 1, or 0.5 hours after the other component(s). There may be a difference in the sequential release rates for each of the components. For instance, rhodamine is delivered during the first 48 hours, followed by FDG during the next 48 hours, and finally by fluoroacetate during the last 48 hours.
- 10 Taking the above mentioned example again, the 12 punctures around a prostate cancer may first be made with a composition comprising a slow release formulation of fluoroacetate. Later on other punctures are performed with a composition comprising a slow release formulation of FDG, if PSA levels do not come down completely after the first therapy. Later still, other punctures are performed with a third composition comprising a slow release
- 15 formulation of rhodamine 123 if PSA levels do not come down completely after the second therapy.
- In sequential administration, one component may be administered once, or any number of times and in various doses before and/or after administration of another component.
- 20 Sequential administration may be combined with simultaneous or sequential administration.
- Derivatives*
- Stereoisomer, tautomers, racemates, prodrugs, metabolites, pharmaceutically acceptable salts, bases, esters, structurally related compounds or solvates of TCA cycle, glycolysis or
- 25 oxidative phosphorylation inhibitors are within the scope of the invention.
- The pharmaceutically acceptable salts of the compounds according to the invention, i.e. in the form of water-, oil-soluble, or dispersible products, include the conventional non-toxic salts or the quaternary ammonium salts which are formed, e.g., from inorganic or organic acids or bases. Examples of such acid addition salts include acetate, adipate, alginate,
- 30 aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate,
- 35 methanesulfonate, 2-naphthalenesulfonate, nicotinate, oxalate, pamoate, pectinate,

persulfate, 3-phenylpropionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, tosylate, and undecanoate. Base salts include ammonium salts, alkali metal salts such as sodium and potassium salts, alkaline earth metal salts such as calcium and magnesium salts, salts with organic bases such as dicyclohexylamine salts, N-methyl-D-glucamine, and salts with amino acids such as arginine, lysine, and so forth. Also, the basic nitrogen-containing groups may be quaternized with such agents as lower alkyl halides, such as methyl, ethyl, propyl, and butyl chloride, bromides and iodides; dialkyl sulfates like dimethyl, diethyl, dibutyl; and diamyl sulfates, long chain halides such as decyl, lauryl, myristyl and stearyl chlorides, bromides and iodides, aralkyl halides like benzyl and phenethyl-bromides and others. Other pharmaceutically acceptable salts include the sulfate salt ethanolate and sulfate salts.

The term "stereoisomer", as used herein, defines all possible compounds made up of the same atoms bonded by the same sequence of bonds but having different three-dimensional structures which are not interchangeable, which the compounds of the present invention may possess. Unless otherwise mentioned or indicated, the chemical designation of a compound herein encompasses the mixture of all possible stereochemically isomeric forms, which said compound may possess. Said mixture may contain all diastereomers and/or enantiomers of the basic molecular structure of said compound. All stereochemically isomeric forms of the compounds of the invention either in pure form or in admixture with each other are intended to fall within the scope of the present invention.

The compounds according to the invention may also exist in their tautomeric forms. Such forms, although not explicitly indicated in the compounds described herein, are intended to be included within the scope of the present invention.

For therapeutic use, the salts of the compounds according to the invention are those wherein the counter-ion is pharmaceutically or physiologically acceptable.

As used herein and unless otherwise stated, the term "solvate" includes any combination which may be formed by a compound of this invention with a suitable inorganic solvent (e.g. hydrates) or organic solvent, such as but not limited to alcohols, ketones, esters and the like.

The term "pro-drug" as used herein means the pharmacologically acceptable derivatives such as esters, amides and phosphates, such that the resulting *in vivo* biotransformation

product of the derivative is the active drug. The reference by Goodman and Gilman (*The Pharmacological Basis of Therapeutics*, 8th Ed, McGraw-Hill, Int. Ed. 1992, "Biotransformation of Drugs", p 13-15) describing pro-drugs generally is hereby incorporated. Pro-drugs of the compounds of the invention can be prepared by modifying functional groups 5 present in said component in such a way that the modifications are cleaved, either in routine manipulation or *in vivo*, to the parent component. Typical examples of pro-drugs are described for instance in WO 99/33795, WO 99/33815, WO 99/33793 and WO 99/33792 all incorporated herein by reference. Pro-drugs are characterized by increased bio-availability and are readily metabolized into the active inhibitors *in vivo*. Specific examples of prodrugs 10 comprising cholesterol or vitamin A are described below.

Administration
Administering to the region of the proliferation avoids or minimises peripheral toxicity, and permits delivery of efficacious doses. It also avoids toxicity associated with oral or systemic 15 delivery. Methods of local delivery are known in the art.

According to one embodiment of the invention, the composition is administered into the proliferating tissue. The composition can enter the tissue, for example, by puncturing the surface of the tumour and injecting composition therein. Such administration is achieved by 20 positive pressure application, for example, by high or low pressure injection, etc as described below. Alternatively, it may be achieved by intra-arteria infusion as also described below. Alternatively, the composition may enter the tissue after opening, for example, by resection surgery to remove the proliferating cell mass, as elaborated below.

25 According to one embodiment of the invention, a composition is administered under positive pressure to a proliferating tissue. Devices suitable for pressured delivery are known in the art, for example from US 2001/0034503 which is incorporated herein by reference. The latter document describes an apparatus for injecting a composition into a tissue under high pressure through a thin catheter (200 microns outer diameter for instance) with very small 30 lateral holes (50 microns) located at the level of the tissue. One may leave the catheter inside the proliferating tissues for several days, and inject determined amounts of substances directly inside the proliferating structure at regular intervals allowing effective penetration of tissue. Where the proliferating cells form a large tissue mass, for example, a pressure between 2 to 5000 Atm. may used to apply the composition, leading to effective 35 distribution of several centimetres radius within the tissue mass. Such injections are rapidly

administered in a single shot. As the injected substances may be very toxic (e.g. fluorocitrate, arsenate, cyanide), it is important to be able to modulate the injected amounts very precisely as it is the case with this device. According to an aspect of the invention, a quantity of composition between 1 microlitre and 100 ml is injected at a pressure between 5 100 and 2500 Atm. The treatment may be repeated at defined intervals or as necessary.

According to another embodiment of the invention, a composition is delivered locally, preferably into the proliferating cell mass, under pressure by means of a continuous pump. A pump may be used to inject composition over a period of time, from several hours to several 10 days as necessary. A pump may take the form of a mechanically operated syringe.

According to another embodiment of the invention a composition is delivered intra-arterially using protracted infusion. Use of intra-arterial protracted infusion allows the composition to infuse directly into the proliferating structure, via a feeding artery. A catheter may be left in 15 place for several minutes to months in order to deliver the therapy efficiently.

According to one embodiment of the invention, a composition is administered under positive pressure to a proliferating tissue by directed injection into the tissue, for example using a needle and syringe.

20 According to another embodiment of the present invention, the composition is administered by injection or deposition into a resection cavity or scar or area of proliferating cells. Thus resection cavities after surgical debulking of tumours can be treated. For example, said cavity any of brain tumour resection, breast tumour resection, prostate cancer resection, 25 muscle resection after a sarcoma, uterine laparoscopic myoma resection, head and neck resection cavities, tongue tumour resection, partial upper maxillar resection, liver tumour resection, kidney tumour resection, or bone tumour resection, scar cavity of a melanoma resection. Furthermore, the bed of cheloid scars after resection may be treated by the present composition, in order to avoid cheloid or hypertrophic scar formation in the 30 population known at risk for such reactions.

According to another embodiment of the present invention, said composition is injected into a mass of proliferating cells such as a tumour under visual control, using ultrasound, MRI, CT-scan, PET-CT or any other imaging means. The authors have found that intratumour 35 diffusion is optimal when there is an homogeneous coverage of the whole tumour volume,

and that an injection performed tangentially to the tumour volume is less efficacious. Such mode of injection may be performed using a syringe, pump or high pressure as described above.

- 5 Administering a composition of the present invention in a proliferating cell mass not only treats the cell mass but also the lymphatic system through which the tumour drains. With conventional therapies, such as radiotherapy the lymphatic pathways are destroyed. With systemic chemotherapy, there is no selective treatment of the lymphatic pathways. In the present invention, by contrast, both the lymphducts and lymph nodes are selectively treated,
- 10 in addition to the cellular proliferation.

- *Slow release formulation*

According to another embodiment of the invention, a slow release formulation of the composition is delivered by application, injection or puncture to or proximal to the site of proliferation. Preferably it is administered into a mass of proliferating cells. The slow release agent regulates, slows the release of inhibitor from the composition. A single dose can comprise a large or concentrated dose, which, once at the site of proliferation is released at a rate determined by the formulation. This avoids the need for prolonged treatment times and a frequency of administration. Another advantage of a slow release formulation is that the composition diffuses day and night, over several days or weeks. The inhibitors can act when a patient is fasting, (e.g. every night) and there is no competition from the degradation products of ingested meals.

- 25 The present inventors have found that inhibitor uptake can be relatively slow in some tumours by observing ¹⁸F-FDG and ¹¹C-acetate by PET-CT. Although tumours may show as an intense signal due to the sensitivity of the imaging and probe, this is deceptive of the uptake rate which can be relative low e.g. in the range of ng/min. Consequently, a slowly releasing inhibitor is better able to match the rate of inhibitor take by the tumour, and avoid
- 30 wasteful and toxic overdosing.

For instance, a slow release formulation of a composition comprising fluoroacetate, rhodamine 123 and 2-Fluoro-deoxy-glucose, and, for example, polyorthoester polymer may be applied on a cervical area, if a cervical dysplasia is diagnosed. Pathologic areas are 35 usually detected using acetate applied on the cervix; with dysplastic areas indicating a colour

change after this application. In the present case, the composition adheres to the cervix and is actively absorbed by the transformed cells. The cells are destroyed, avoiding the need for a superficial laser therapy.

- 5 One embodiment of the present invention is a composition as described herein, further comprising one or more slow release agents. Slow release agents may be natural or synthetic polymers, or reabsorbable systems such as magnesium alloys.

Among the synthetic polymers useful according to a slow release formulation of the invention
10 are poly(glycolic) acid, poly(lactic acid) or in general glycolic- and lactic acid based polymers and copolymers. They also include poly caprolactones and in general, poly hydroxyl alkanoates (PHAs) (poly(hydroxy alcanoic acids) = all polyester). They also include Poly (ethylene glycol), poly vinyl alcohol, poly (orthoesters), poly (anhydrides), poly (carbonates), poly amides, poly imides, poly imines, poly (imino carbonates), poly (ethylene imines),
15 polydioxanes, poly oxyethylene (poly ethylene oxide), poly (phosphazenes), poly sulphones, lipids, poly acrylic acids, poly methylmethacrylate (PMMA), poly acryl amides , poly acrylo nitriles (Poly cyano acrylates), poly HEMA, poly urethanes, poly olefins, poly styrene, poly terephthalates, poly ethylenes, poly propylenes, poly ether ketones, poly vinylchlorides, poly fluorides, silicones, poly silicates (bioactive glass), siloxanes (Poly dimethyl siloxanes),
20 hydroxyapatites, lactide-capronolactones and any other synthetic polymer known to a person skilled in the art.

Among the natural derived polymers useful according to a slow release formulation of the invention, are poly aminoacids (natural and non natural), poly β -aminoesters. They also
25 include poly (peptides) such as: albumines, alginates, cellulose / cellulose acetates, chitin / chitosan, collagene, fibrine / fibrinogen, gelatine, lignine. In general, proteine based polymers. Poly (lysine), poly (glutamate), poly (malonates), poly (hyaluronic acids). Poly nucleic acids, poly saccharides, poly (hydroxyalkanoates), poly isoprenoids, starch based polymers, and any other natural derived polymer known to a person skilled in the art.

30 Other polymers may be made from hydrogels based on activated polyethyleneglycols (PEGs) combined with alkaline hydrolyzed animal or vegetal proteins.

For both synthetic and natural polymers, the invention includes copolymers thereof are included as well, such as linear, branched, hyperbranched, dendrimers, crosslinked, functionalised (surface, functional groups, hydrophilic/hydrophobic).

- 5 The slow release composition may be formulated as liquids or semi-liquids, such as solutions, gels, hydrogels, suspensions, lattices, liposomes; or implants, such as particles, films, rods, fibres, grains. Solid or semi-solid formulations such as rods, fibres or grains improve the ease of administering the implant. Semi-liquid, liquid substance or polymer, or active substance presenting as powder, may be encapsulated in a resorbable capsule or
10 tube made from gelatine, or any polymer degrading rapidly. Any suitable formulation known to the skilled man is within the scope the scope of the invention. According to an aspect of the invention, a composition is formulated such that the quantity of inhibitor is between less than 1% and 60 % of total slow-release polymer mass. According to an aspect of the invention, a composition is formulated such that the quantity of inhibitor is between 1% and
15 50%, 1% and 40%, 1% and 30%, 1% and 20%, 2% and 60%, 5% and 60%, 10% and 60%, 20% and 60%, 30% and 60%, or 40% and 60% of total slow-release polymer mass.

Where the slow release agent has the properties to form a semi-solid (gel-like) polymer, the composition may take the form of a foil allowing the release of inhibitor in a controlled
20 fashion, for instance in contact with superficial skin cancers. The foil administers composition directly into the cells owing to the properties of the hydrogel; the high water content of the gel creates a fully communicating structure to the interior of the proliferating mass of cells. Oncotic pressure holds water inside hydrogel and attracts waters from skin, and makes easy transfer of molecules from gel to skin.

25 One example of such foil may be, for example, a polymer made from hydrogels based on activated polyethyleneglycols (PEGs) combined with alkaline hydrolyzed soya solutions or other animal or vegetal proteins (bovine serum albumin, soya globulin, casein, pea albumin, starch albumine, ovalbumin, etc).

30 Such a foil could be for instance 3 mm thick and be filled with saline water to a percentage of 90 % or more, containing one to several ATP inhibitors.

35 Such foils may be used easily to treat superficial skin cancers. For instance, squamous cell skin cancers or basal carcinomas present usually with a round shape, for instance, 1, 3, or 5

cm in diameter. When in contact with the superficial skin tumour, such a hydrogel foil hydrates actively the skin, humidifies the epithelium, and allows easy transfer of inhibitor(s) to the superficial tissues of the tumour.

- 5 The typical load of such a 3 mm thick hydrogel would be in the range of 10 mg of glycolysis inhibitor per square cm of hydrogel or less, in the range of 0.1 mg of TCA inhibitor per square cm of hydrogel or less and in the range of 1 mg oxidative phosphorylation inhibitor per square cm of hydrogel or less.
- 10 Once in contact with the lesion, the hydrogel releases slowly the inhibitor(s) inside the superficial tumour.

Typically, the major part of each inhibitor will be delivered to the lesion in a period of 4 to 8 hours. The hydrogel may be replaced every day, until the lesion disappears, which should

15 happen within 1 or 2 weeks.

The lesion may be treated in addition using radiotherapy. The standard doses for the treatment (typically 10 times 4 Gy) could be reduced by 20 to 50 %.

20

Solubilising agents

According to another embodiment of the present invention at least one TCA cycle inhibitor, at least one glycolysis inhibitor and/or at least one inhibitor of oxidative phosphorylation of a composition is coupled to one or more solubilising agents. Such agents change the hydrophilic and hydrophobic profile of the inhibitor, depending on the required solubility. For example, if a composition according to the invention comprises a hydrophilic TCA cycle inhibitor such as fluoroacetate, and a hydrophobic slow release polymer such as polyorthoester, the inhibitor will not adequately solubilise or suspend within the composition. Similarly, a composition according to the invention comprising a very hydrophilic glycolysis inhibitor such as 2-FDG, will lead to an inadequately solubilised or emulsified composition. Consequently the release properties of the slow release agent may be compromised, and degradation within the body accelerated. To overcome this, the inventors have coupled at least one TCA cycle inhibitor, at least one glycolysis inhibitor and/or at least one oxidative phosphorylation inhibitor to a solubilising agent which changes the hydrophobicity or hydrophilicity of the inhibitor, depending on the required formulation. The composition so

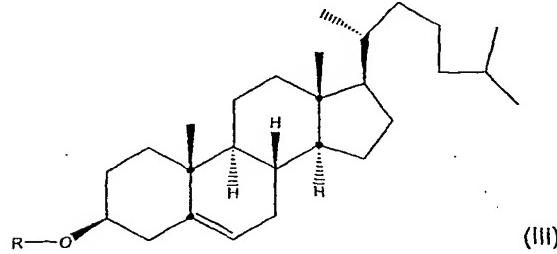
formed is more stable. According to one aspect of the invention, the coupled compound is a prodrug wherein the solubilising agent is cleaved *in vivo*, so releasing the inhibitor. According to another aspect of the invention, the solubilising agent is cleaved from the inhibitor more rapidly by the proliferating cells.

5

- Cholesterol

According to one aspect of the invention, cholesterol (III) or a derivative thereof is a coupling agent. One embodiment of the invention is a composition as mentioned herein in which at least one TCA cycle inhibitor, at least one glycolysis inhibitor and/or at least one oxidative phosphorylation inhibitor is coupled to cholesterol (III) or derivatives thereof.

10



15

wherein R may be one of the following substances: betahydroxybutyrate, halogenated butyrate, halogenated acetate, halogenated aceto-acetate, halogenated acetamide, halogenated crotonate, halogenated acetone, halogenated citrate and halogenated oleate..

Derivatives of cholesterol are modifications which retain or enhance of activity of the parent compound. Derivatives include, but are not limited to cholesteryl-3-betahydroxybutyrate, 20 cholesteryl-halogenated butyrate, cholesteryl-halogenated acetate, cholesteryl-halogenated aceto-acetate, cholesteryl-halogenated acetamide, cholesteryl-halogenated crotonate, cholesteryl-halogenated acetone, cholesteryl-halogenated citrate, or cholesteryl-halogenated oleate.

25 Halogenated means fluoro-, chloro-, bromo- or iodo-modified.

An advantage of using cholesterol or a derivative thereof as a solubilising agent is such natural metabolite can enter a cell via a number of mechanisms including through the lipid bilayer of the cell membrane. In rapidly proliferating cells, absorption is more rapid due to the

requirement for cholesterol in cell membranes. Once in the lipid bilayer, flippase enzyme transfers the cholesterol-coupled inhibitor from the outer layer to the inner layer; cholesterol is internalised in the cytosol and the inhibitor is released from cholesterol by cholesterol-metabolising enzymes.

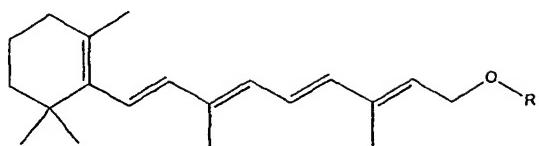
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A cholesterol molecule is coupled to an inhibitor using known methods. For example, coupling may proceed via nucleophilic attack by electrons of an oxygen atom on the cholesterol. For example, coupling may proceed via nucleophilic attack by electrons of an oxygen atom on the inhibitor. According to another example, esters, ethers or other 10 derivatives of cholesterol or inhibitor may be prepared to facilitate coupling. Mechanisms and knowledge of appropriate coupling moieties are known to the skilled person for the preparation of such coupled inhibitors.

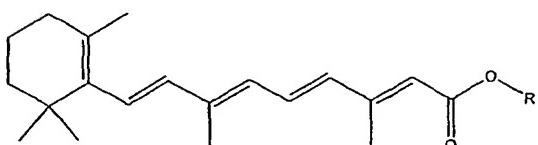
One embodiment of the present invention is a composition comprising cholesteryl-fluorobutyrate, 2-FDG, dinitrophenol and polyorthoester.

- Vitamin A

According to one aspect of the invention, vitamin A (retinol) or a derivative thereof is a coupling agent. One embodiment of the invention is a composition as mentioned herein in 20 which at least one TCA cycle inhibitor, at least one oxidative phosphorylation inhibitor and/or at least one glycolysis inhibitor is coupled to vitamin A or derivatives thereof. Examples of derivatives include the ether (IV) and ester (V) forms which groups facilitate ease of coupling:



(IV)



(V)

wherein R may be one of the following substances: betahydroxybutyrate, halogenated butyrate, halogenated acetate, halogenated aceto-acetate, halogenated acetamide, 5 halogenated crotonate, halogenated acetone, halogenated citrate, halogenated oleate.

Derivatives of vitamin A are modifications which retain or enhance of activity of the parent compound. Derivatives include, but are not limited to those mentioned above and betahydroxybutyrate, halogenated butyrate, halogenated acetate, halogenated aceto- 10 acetate, halogenated acetamide, halogenated crotonate, halogenated acetone, halogenated citrate, or halogenated oleate.

Halogenated means fluoro-, chloro-, bromo- or iodo-modified.

15 An advantage of using vitamin A or a derivative thereof as a solubilising agent is that such natural metabolite can enter a cell via a number of mechanisms. In rapidly proliferating cells, absorption is more rapid, especially in vitamin A metabolising cells such as found in liver tissue. The effect may be used to treat, for instance, hepatocarcinomas by injecting a slow release polymer of retinyl ether or retinoic acids ester coupled with haloacetates directly 20 inside the hepatocarcinoma mass. The antiproliferative effect commences once the inhibitor is liberated from the polymer and vitamin A is metabolised.

A vitamin A is coupled to an inhibitor using known methods. For example, coupling may proceed via nucleophilic attack by electrons of an oxygen atom on the vitamin A. For example, coupling may proceed via nucleophilic attack by electrons of an oxygen atom on the inhibitor. According to another example, esters or other derivatives of vitamin A or 5 inhibitor may be prepared to facilitate coupling. Mechanisms and knowledge of active groups are known to the skilled person for the preparation of such coupled inhibitors

One embodiment of the present invention is a composition comprising vitamin A-fluoroacetate, dinitrophenol, 2-FDG and polyorthoester.

10

Encapsulated inhibitor

According to one aspect of the invention at least one TCA cycle inhibitor, at least one glycolysis inhibitor and/or at least one oxidative phosphorylation inhibitor of a composition is encapsulated in one or more micro-capsules or nano-capsules.

15

Examples of nano-capsules (or nano-spheres) or formulations therewith include, but are not limited to a copolymer poly(ethylene oxide) with poly(L-Lactic acid) or with poly(beta-benzyl-L-aspartate); copolymer with poly(lactide-co-glycolide)-[(propylene oxide)-poly(ethylene oxide)]; polyphosphazene derivatives; poly(ethylene glycol) coated nanospheres; 20 poly(isobutylcyanoacrylate) nanocapsules; poly(gamma-benzyl-L-glutamate)/(poly(ethylene oxide)); chitosan-poly(ethylene oxide) nanoparticles; nanoparticles where the anti-proliferative drug is prepared using o-carboxymethylate chitosan (o-CMC) as wall forming material; silicone nanocapsules, solid lipid nanoparticles or nanospheres (SLNs) and any known formulation of nano-particles known to someone skilled in the art.

25

Examples of micro-capsules (or micro-spheres) or formulations therewith include but are not limited to multiporous beads of chitosan; coated alginate microspheres; N-(aminoalkyl) chitosan microspheres; chitosan/calcium alginate beads, poly(adipic anhydride) microspheres; gellan-gum beads; poly(D, L-lactide-co-glycolide) microspheres; alginate-poly-30 L-lysine microcapsules; crosslinked chitosan microspheres; chitosan/gelatin microspheres; crosslinked chitosan network beads with spacer groups; aliphatic polyesters such as 1,5-diozepan-2-one and D,L-dilactide microspheres; triglyceride lipospheres; polyelectrolyte complexes of sodium alginate chitosan; polypeptide microcapsules; albumin microspheres; and any other micro-capsule (or micro-sphere) formulation known to someone skilled in the 35 art.

- By using encapsulated inhibitor, the solubility profile of the inhibitor may be changed according to the environment of the formulation. It may thus act as a solubilising agent as mentioned above. An example of its use is when, an inhibitor of the invention is hydrophilic and a slow-release gel is hydrophobic. An encapsulated inhibitor has an advantage that solubilisation does not require chemical coupling of the inhibitor. Thus, an encapsulated inhibitor allows solubility or emulsification in the slow-release agent, so preventing an otherwise unstable formulation.
- 10 Furthermore, encapsulation may be used to modulate the release by the slow-release agent (e.g. fine tune or prolong release time). Furthermore, encapsulation may be used to improve intracellular penetration, as known for encapsulations such as SLN. The advantages of encapsulated formulation may be applied to inhibitor already chemically modified to improve solubility. For example, cholesterol coupled fluoroacetate may be prepared in microcapsules 15 within a slow release gel. The formulation so produced would provide solubility for the inhibitor, slow release modulated by the presence of capsules and active cellular penetration.
- Furthermore, encapsulation may be used to modulate release of the inhibitor when a slow-release agent is not present in a composition. For example, when an inhibitor is administered 20 by infusion or injected under high pressure, the composition may comprise one or more TCA cycle inhibitors in presence of micro- or nano-capsules, one or more glycolysis inhibitors, and one or more oxidative phosphorylation inhibitors in presence of micro- or nano-capsules. Such composition may reduce the frequency and/or duration of treatment compared with conventional formulations.
- 25 One embodiment of the present invention is a composition as mentioned above in which at least one inhibitor is encapsulated in micro- or nano-capsule(s) (or micro- or nano-sphere(s)). According to one aspect of the invention, at least one inhibitor is also pre-coupled to a solubilising agent as mentioned above.
- 30
- Solid wall composition*
- In cases where the composition is viscous, such as, for example, when a particular slow-release agent is present, it can be difficult to administer into a proliferating mass owing to the 35 force required to move the composition through administering tubing or needle. To make

administration easier, the composition may be formed into one or more solid wall entities *i.e.* entities having at least solid or semi-solid walls, which entities are small enough to pass through a needle and into the proliferating mass.

- 5 According to one aspect of the invention, a solid wall composition is where the composition is enclosed within a solid or semi-solid bioabsorbable membrane to form a contained capsule. Such capsules are of suitable size and shape (small enough) to pass through a needle and into the proliferating mass. Once administered, the capsule dissolves, and the composition is released. Optionally, the composition may also be disposed on the exterior surface of the
 - 10 capsule, and/or impregnated within the membrane of the capsule. The capsule can be spherical, oval, seed-shaped, tubular or any suitable shape for administration using a needle. The capsule can be made of any suitable biocompatible and bioabsorbable material such as, for example, gelatine.
 - 15 According to another aspect of the invention, a solid wall composition is a solid state bioabsorbable structure, impregnated with composition. Such solid state structures are of suitable size and shape (small enough) to pass through a needle and into the proliferating mass. Once administered, the structure dissolves, and the composition is concomitantly released. The structure can be seed-shaped, rod-shaped, tube-shaped or any suitable shape
 - 20 for administration using a needle. The structure can be made of any suitable biocompatible and bioabsorbable material such as, for example, aliphatic polyesters such as homopolymers and copolymers of lactic acid, glycolic acid, lactide, glycolide, para-dioxanone, trimethylene carbonate, epsilon-caprolactone, lactide-capronolactone etc. and blends thereof.
- 25 The solid wall composition readily passes through tubing, requiring less force compared with liquid viscous compositions. Furthermore, the precise dose of composition can be administered, and no residual composition is left in the syringe or needle. The solid wall composition can be administered individually, as a series of punctures, for example, one rod per injection. Or, where sufficiently small (nanocapsules), administered in a similar manner to a liquid composition. The maximum width of a solid wall composition is less than the internal diameter of the administering tubing or needle, and can be less than 3 mm, 2 mm, 1 mm, 0.5 mm or a width in the range between any two of the aforementioned widths.

Bone metastases

Another embodiment of the present invention is a composition as described herein further comprising one or more pyrophosphates. Said composition may be used to treat a bone 5 tumour first, by preventing tumour cell proliferation, and in a second step, to stimulate bone reconstruction. The composition may be injected inside a bone metastasis. According to the present invention pyrophosphate may be any suitable salt of pyrophosphate, including, but not limited to sodium pyrophosphate, potassium pyrophosphate, calcium pyrophosphate. The pyrophosphates may be mixed to the polymer containing the inhibitors. Once the 10 polymer has been degraded and all inhibitors have been absorbed, the presence of pyrophosphates may stimulate new bone formation.

Separate, simultaneous or sequential

15 According to one aspect of the invention, pyrophosphate may be administered simultaneously, separately or sequentially in respect of the other components of the composition of the invention.

Another aspect of the invention is a composition comprising pyrophosphate, at least one 20 TCA cycle inhibitor, at least one glycolysis inhibitor and at least one inhibitor of oxidative phosphorylation as disclosed herein, for simultaneous, separate or sequential administration to a subject.

One aspect of the invention is a method for treating cellular proliferation comprising 25 administering to an individual an effective amount of pyrophosphate and at least one TCA cycle inhibitor of the invention and/or at least one glycolysis inhibitor, and/or at least one cycle inhibitor of oxidative phosphorylation, simultaneously, separately or sequentially.

By simultaneous administration means the pyrophosphate, and at least one inhibitor of TCA 30 cycle, glycolysis and oxidative phosphorylation are administered to a subject at the same time. For example, as a mixture or a composition comprising said components. An example is as a solution comprising the pyrophosphate, TCA cycle inhibitor, glycolysis inhibitor and oxidative phosphorylation inhibitor.

By separate administration means the pyrophosphate and at least one inhibitor are administered to a subject at the same time or substantially the same time. The components may be present in a kit as separate, unmixed preparations. For example, the pyrophosphate, TCA cycle inhibitor, glycolysis inhibitor and oxidative phosphorylation inhibitor may be

- 5 present in the kit as individual vials. The inhibitors may be administered to the subject by separate injections at the same time, or injection directly following the other.

By sequential administration means the pyrophosphate and at least one inhibitor are administered to a subject sequentially. The pyrophosphate, TCA cycle inhibitor, glycolysis

- 10 inhibitor and oxidative phosphorylation inhibitor may be present in a kit as separate, unmixed preparations, for example. There is a time interval between doses. For example, one component might be administered up to 336, 312, 288, 264, 240, 216, 192, 168, 144, 120, 96, 72, 48, 24, 20, 16, 12, 8, 4, 2, 1, or 0.5 hours after the other component.

- 15 In sequential administration, one component may be administered once, or any number of times and in various doses before and/or after administration of another component. Sequential administration may be combined with simultaneous or sequential administration.

Combined radiotherapy or chemotherapy treatment

- 20 Another aspect of the invention, is a method of treating proliferating cells comprising delivering to proliferating cells a composition according to the invention, and radiotherapy and/or chemotherapy. The use of the composition can lead to effective treatment using a fraction of the normal radiotherapy or chemotherapy therapeutic dose.

- 25 According to this aspect of the invention, proliferating cells are treated by administering a composition locally, preferably into the proliferating cell mass, as mentioned above. According to this aspect of the invention, a tumour is totally or partially resected and an implant is placed inside the resection cavity as mentioned above. The site of the proliferation
30 is then treated with radiotherapy applied either from an exterior source, or by the manual or automatic insertion of radioactive sources (brachytherapy). The radioactive source may also be a radio-isotope-halogen (RIH) inhibitor as described above. The combination of locally administered composition and radiotherapy treatments may lead to a rapid and effective shrinking or death of the proliferation because it renders the tumour cells much more
35 sensitive.

- According to this aspect of the invention, proliferating cells are treated by administering a composition locally, preferably into the proliferating cell mass, as mentioned above, and in addition, the site of the proliferation is treated with intravenous chemotherapy (for instance paclitaxel, cisplatin, vinorelbine, etc). Alternatively, a tumour is totally or partially resected and an implant is placed inside the resection cavity as mentioned above as mentioned above, and in addition, the site of the proliferation is treated with intravenous chemotherapy. The combination of locally administered composition and radiotherapy and/or chemotherapy treatments may lead to a rapid and effective shrinking or death of residual tumour or tumour cells. It is expected that chemotherapy and/or radiotherapy will be much more efficient after the local application of the inhibitors inside the proliferating process. It is foreseen that accumulated doses of radiotherapy and/or chemotherapy could be decreased by 10 to 50 %.
- 15 One embodiment of the present invention is a method for treating cellular proliferation comprising administering a composition locally, preferably into the proliferating cell mass, as described herein in combination with radiotherapy.
- One embodiment of the present invention is a method for treating cellular proliferation comprising administering a composition locally, preferably into the proliferating cell mass, as described herein in combination with chemotherapy.
- 20 Another embodiment of the present invention is a method for reducing the dose of radiotherapy treatment of a tumour, comprising administering a composition locally, preferably into the proliferating cell mass, as mentioned above prior to radiotherapy.
- 25 Another embodiment of the present invention is a method for reducing the dose of chemotherapy treatment of a tumour, comprising administering a composition locally, preferably into the proliferating cell mass as mentioned above prior to chemotherapy.
- 30 Where radiotherapy and chemotherapy are administered, the composition of the present invention may be used to reduce both radiotherapy and chemotherapy doses. A typical chemotherapy and/or radiotherapy dose may be about 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% less than the dose normally applied to a tumour, in view of the size, location and

other factors. It may be a value in the range between any two of the aforementioned values. Preferably, the dose is between 20 and 70% less than the normal dose.

Another embodiment of the present invention is a method for sensitising a proliferating cell mass (e.g. tumour) to radiotherapy, comprising administering a composition locally, preferably into the proliferating cell mass as mentioned above prior to radiotherapy.

Another embodiment of the present invention is a method for sensitising a proliferating cell mass (e.g. tumour) to chemotherapy, comprising administering a composition locally, preferably into the proliferating cell mass as mentioned above prior to chemotherapy.

According to one aspect of the invention, the composition is applied to a subject at least 8 hours, 10 hours, 12 hours, 14 hours, 16 hours, 18 hours, 20 hours, 22 hours, 1 day, 2 days, 3 days, 4 days, 6 days, 8 days, 10 days, 12 days, 14 days, 3 weeks or 4 weeks before the start of radio- and/or chemotherapy, or for a period between any two of the aforementioned periods. Preferably, the composition is in place for between 12 hours to 4 weeks before commencement of radio- and/or chemotherapy.

20 Dose

The quantity and concentration of composition for injection and the frequency of administration can be calculated using known techniques by the skilled person.

Typically, 1 to 3-4 grams of the composition may be administered to a 30-50 gram tumor. The injections may be made in several places inside or proximal to the tumor, in order to warrant an homogeneous distribution of the substances. For instance a 30 gram cervical lymphnode from a tonsillar tumor would receive under ultrasonic control 3 injections of 1 gram of composition in 3 different places of the lymphnode. Each gram of the composition would comprise 0.5-2 milligrams of fluoroacetate, 3 milligrams of dinitrophenol and 30-50 mg of oxamate, for instance. If the composition comprises a tri-block copolymer with a release period of 1 month, the patient is re-examined after 1 month. If the lymphnode did not disappear, but has shrunk to half of its initial size, 1 to 2 injections of 1 gram of the polymer formulation may be repeated 1 month after the first injection, and again 1 month later if necessary to kill all tumor cells.

The active substance may be deposited as a narrow (e.g. 1 to 1.45 mm diameter) cylinder of paste extruded from a needle or tube into the proliferating mass. The polymer will slowly release the product. If several such cylinders are deposited inside a lesion, it ensures a better homogeneity for the therapy. A person skilled in the art will take into account the 5 release rate of the polymer (for instance 5 days or 6 weeks), in order to clinically observe the effects of the treatment. After all the drug has been released, the effect should be considered as being maximal. A repeated therapy will be decided if necessary.

Some cancers are present as huge masses, for instance 5 kgs or 18 kgs. In these cases, the 10 local therapy could be performed –when possible- as a intra-arterial infusion first, in order to decrease the tumoral volume. When the volume has decreased, after several weeks, the tumor may be implanted with a slow-release polymer containing an active substance in a second step.

15 According to one aspect of the invention, a composition comprises TCA inhibitor such that the inhibitor concentration delivered to a subject is greater than or equal to 1, 10, 20, 40, 60, 80, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000 or mg inhibitor / kg of tumour or of treated mass, or a concentration in the range between any two of the aforementioned values. Preferably the dose is between 1 and 200 mg/kg of tumour or of treated mass.

20 According to one aspect of the invention, a composition comprises glycolysis inhibitor such that the inhibitor concentration delivered to a subject is greater than or equal to 20, 40, 60, 80, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500, 2000, 2500, 3000 or mg inhibitor / kg of tumour or of treated mass, or a concentration in the range between any two 25 of the aforementioned values. Preferably the dose is between 0.02 and 2 g/kg of tumour or of treated mass.

According to one aspect of the invention, the composition comprising oxidative phosphorylation inhibitor in an amount such that the concentration of inhibitor delivered to a 30 subject is greater than or equal to 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 or 60, 500, 1000, 1500, 2000 mg inhibitor / kg, or a concentration in the range between any two of the aforementioned values. Preferably the dose is between 0.010 and 2 g/kg of tumour or of treated mass.

According to one aspect of the invention, a composition comprises an amount of rhodamine to deliver a concentration in the range 0.5 to 3 g/kg, 1 to 2.5 g/kg, and preferably 1.25 to 2.0 g/kg of tumour or of treated mass. According to another aspect of the invention, a composition comprises an amount of rhodamine to deliver a concentration in the range 0.5 to 5 2 g/kg, 0.75 to 1.75 g/kg, and preferably 1 to 1.5 g/kg of tumour or of treated mass, when used in combination with cytotoxic therapy such as chemo- or radiotherapy.

Imaging agents

- 10 Another embodiment of the present invention is a composition further comprising one or more imaging agents which allow the composition to be viewed using an *in vivo* imaging device. According to one aspect of the invention, a composition further comprises a magnetic-resonance-visible agent, such as, for example, a MR visible polymer such as a poly(ortho)ester, metallic powder (e.g. tantalum powder), or any other MR visible agent.
- 15 According to one aspect of the invention, a composition further comprises a radio-opaque agent, such as, for example, biocompatible metal powder (e.g. tantalum, iridium powder, magnesium alloy powder), or any other agent appear opaque to X-rays. According to another aspect of the invention, a composition further comprises micro-bubbles in order to render the composition ultrasound visible.
- 20 The addition of imaging agents allows a physician to accurately administer the composition with the assistance of an *in vivo* imaging device, and also to follow the distribution in the tumour mass thereafter.
- 25 *Combinations*
- A composition comprising one or more of the aforementioned components is within the scope of the present invention. For example, a composition may comprise one or more TCA cycle inhibitors optionally coupled to solubilising agent, one or more glycolysis inhibitors optionally coupled to solubilising agent, one or more oxidative phosphorylation inhibitors 30 optionally coupled to solubilising agent, one or more slow-release agents, one or more pyrophosphates, one or more micro- or nano-particles containing at least one of the active substances, one or more one or more sensitising agents, and/or one or more imaging agents, and other components known to the skilled person for suitable formulation of the composition.

The inventors have also found that inhibition of oxidative phosphorylation, glycolysis and TCA cycle is effective against proliferating cells; proliferating cells do not obtain energy via other pathways and recover. *In vitro* data shows separate administered inhibitors inhibit growth of cancer cells growth, *in vivo*, given by intravenous route they are not efficaceous to
5 treatment tumours such as prostate cancer. Inhibitors of oxidative phosphorylation, glycolysis and TCA cycle can be extremely toxic to a subject and have been largely overlooked for effective treatment of conditions such as cancer. The doses needed for efficacy via the systemic route, such inhibitors would be very high. By locally delivering a combination of inhibitors into proliferating cells in a slow release formulation, the delivery period is prolonged
10 i.e. the inhibitors are not cleared by the liver, and the dose received by the tumour is effectively higher than using systemic delivery. Furthermore, the lethal dose can be greatly exceeded. Because of the high metabolic rate of proliferating cells, certain inhibitors are rapidly and selectively taken up the proliferating cells and not by the healthy cells. Thus, a composition comprising the present inhibitors in a slow release formulation increases the
15 effective dose to the tumour and permits greater than lethal dosing. Furthermore, the combination of inhibitors leads to a surprisingly effective inhibition of proliferation.

Furthermore, the composition may be used in combination with radiotherapy, where a lower dose of inhibitor may be used.
20

It is possible to select which pathway of glycolysis or TCA is better to be shut down by performing a 2-FDG and a ¹¹C-acetate positron emission tomography examination, and evaluating the activity of glycolysis and TCA cycles in the said tumour, allowing to choose for each individual tumour which compound should be favoured for the inhibition.

25 The combination of ATP inhibitors is very important for the inventors. The inventor's own clinical experience (MR spectroscopy and PET-CT examinations) as well as data from the literature confirm the great variety of substrates taken up by tumours. A review of the literature data shows for instance that the affinity for 18-FDG intake varies from 3 to 100 %,
30 depending on evaluated tumours and affected organs (e.g. http://www.petscaninfo.com/zportal/portals/phys/clinical/jnmpetlit/index_html/JNM_OncoApps/JNM_Table8/article_elements_view). The inventors have also observed that a beneficial therapeutic approach is to treat several ATP synthesis pathways simultaneously.

Kit

A kit according to the invention comprises at least one composition of the present invention.

- It is an aspect of the invention that a composition is provided in a container. For example, a
5 vial, a sachet, a screw-cap bottle, a syringe, a non-resealable vessel, a resealable vessel. Such containers are any that are suitable for containing a composition. Some active products, for example, are sensitive to light and heat and should be preserved in dark and cold.
- 10 A kit may provide a range of vials containing different compositions with different inhibitors, different combinations of inhibitors, different combinations of slow-release polymers. A kit may comprise a means for administering the composition (e.g. one or more syringes). A kit may facilitate the sequential application of more than one type of composition. A kit may contain instructions for use.

15

EXAMPLES

- The invention is illustrated by the following non-limiting examples. They illustrate the effectiveness of a selection of TCA cycle, glycolysis and oxidative phosphorylation inhibitors
20 described above. The inhibitory properties of the other inhibitors not mentioned in the examples are known, and the skilled person may readily substitute the exemplified inhibitors with equivalent pathway inhibitors such as listed above.

25 Example 1

- A patient has a prostate carcinoma, a PSA level at 10 ng/ml, prostate biopsies showing a Gleason score of 7 and lesions present in both lobes. A fluor-choline PET-CT does not show any pathologic lymphnodes in the pelvis. This patient is a candidate for a local therapy such as surgical resection or external or interstitial radiation therapy. Another alternative is to
30 implant the peripheral area of the prostate under ultrasound, MR or CT control with a slow release formulation comprising fluoroacetate, oxamate and dinitrophenol. The patient undergoes loco-regional anesthesia and is placed in gynecological position under an open MR machine or using ultrasonic control. A mixture of poly(ortho)ester and fluoroacetate is injected inside the prostate, in the prostatic peripheral area, several mm inside the prostate
35 capsule (5 to 10 mm). 1 to 8 mg of fluoroacetate in total in slow release formulation are

injected as 8 peripheral injections. Simultaneously, 6 other injections are performed with a mixture comprising 50 mg of oxamate and 10 mg of dinitrophenol between the injection areas of fluoroacetate. As the poly(ortho)ester (POE) is visualised under MR, one can follow its deposition area, and during following days, the degradation of the POE. The
5 injected POE degrades over 10 to 30 days, depending on the local biological conditions. The fluoroacetate, oxamate and dinitrophenol are actively absorbed by the tumor cells. In the following months the therapeutic effect is observed by measuring the PSA blood levels. In case of tumor persistence, a second or a third therapeutic session is realised. The exact tumor area is determined by MR spectroscopy or PET-CT, and this area is implanted
10 exclusively and repeatedly. It is supposed that the injected drug follow the lymphatic pathways and treats microscopic foci in drainage lymphnodes as well. The patient reports no major side effects of the treatment.

Example 2

15 A female patient presents with a biopsy-proven cervix tumor, 3 cm in diameter. No lymphnodes are seen on the MR or PET-CT examinations. The patient refuses the standard therapeutic therapies which would be: (a) the patient is operated on, and the lymphnodes are removed (b) the patient receives radiation therapy simultaneously with a cisplatin based chemotherapy, or finally. The patient is proposed to enter a pilot feasibility trial and she
20 benefits from one to several injections of a composition comprising fluoroacetate, 2-FDG and dinitrophenol in a slow release formulation. Another possibility is to place a tube with lateral holes inside the cervix canal, and to inject a composition comprising fluoroacetate, dinitrophenol and 2-FDG under high pressure from inside the cervical canal, perpendicularly to cervical canal axis, towards the tumor. After several such injections, the tumor shrinks and
25 disappears. The patient is carefully followed-up with pelvic MRI, for loco-regional recurrence and for a recurrence in the lymphnodes. In case the tumour recurs, a standard therapy using radiation or surgery is still possible.

Example 3

30 A patient presents with a 4 cm diameter pulmonary mass located in the left inferior lobe. The histological analysis has shown an epidermoid carcinoma. Bilateral mediastinal lymphatic extension is seen on CT scan examination and the patient is not considered for a surgical intervention. In order to avoid irradiating the surrounding lung tissue (around the primary tumor), an injection of a lactide-capronolactone slow release composition comprising 1-8 mg
35 of slow release fluoroacetate, 200 mg of 2-FDG and 50 mg of rhodamine 123 is performed

inside the tumoral mass, under CT guidance. This drug is delivered over the next 10 days. In parallel, a chemoradiotherapy is started on the mediastinal lesions. Some of the mediastinal invaded lymphnodes may be implanted as well, if easily reachable under CT guided puncture. The radiation dose needed to sterilize the injected lesions is possibly reduced from
5 70-75 to 40-60 Gy.

Example 4

A patient presents with a cheloid scar. The patient is operated, the cheloid scar is resected, and a catheter is left at the bottom of the scar for several days. After 1-2 weeks, when the
10 sealing process of the scar is underway, a composition of the invention comprising slow release polymer is deposited at the bottom of the resected area using the catheter that was left in place, preventing the cheloid scar formation, which initiates at the bottom of the scar. The composition comprises fluoroacetate, 2-FDG and rhodamine 123 delivered at the bottom of the scar is in the range of several micrograms up to milligrams per inhibitor per cm of scar
15 length.

Example 5

A 70 years old patient has been operated for a 1cm breast cancer. The sentinel node technique did not show any lymphatic invasion. In place of undergoing external beam
20 radiation of the breast, the patient receives an injection of a composition comprising a slow release fluoroacetate (1-8 mg), 2-FDG (200 mg) and rhodamine 123 (50mg), 3 weeks after the surgical excision, inside the surgical scar, under US, CT or MR guidance. This treatment will allow external beam radiotherapy to be avoided, sharply reducing the recurrence rate.
When the myoma did not shrink enough, the injection may be repeated.

25

Example 6

A 33 year old woman presents with a 6 cm diameter myoma, a benign tumor, that grows approximately 2 cm in diameter per year. The standard therapy is the surgical resection of the myoma mass, necessitating a surgical intervention. In the present case, a composition
30 comprising 4 mg of fluoroacetate, 50 mg rhodamine and 200 mg of 2-FDG prepared in a slow release formulation are injected twice, 1 month between injections in the myoma mass, leading to its disappearance after several weeks and avoiding the need for a surgical intervention.

35 **Example 7**

A 44 year old patient presents with a 2 cm long esophageal tumor. The echoendoscopic examination shows a 8 mm thick circumferential tumor. A flexible injector is introduced inside the biopsy channel of the endoscope and 4 punctures are performed all around the esophageal circumference, through angulated punctures, inside the tumoral thickening. 1 to 5 mg of fluoroacetate , 200 mg of oxamate and 50 mg of rhodamine 6G in a slow release formulation are delivered. The patient is then submitted to chemotherapy and radiation. Radiation is delivered at a reduced therapeutic dose of 50 Gy instead of 70 Gy and the tumor is totally eradicated.

10 **Example 8**

A 45 y old woman complaints from uterine bleeding. An MRI examination shows 3 benign myomas located in the uterine wall, 5, 3 and 2.5 cm in diameter. The patient undergoes locoregional anesthesia, and under ultrasonic or MRI control all three lesions are punctured and injected each with 1cc to 3 cc of a composition made from a poly(ortho)ester (POE) 15 polymer, rhodamine 123 (40 mg per cc), fluoroacetate (1-2mg per g of POE) embedded in solid-lipid-nanospheres (SLNs), and 2-FDG (200 mg per cc). After 2 months a new MRI examination is performed which shows that the 2.5 cm lesion has nearly disappeared, and the 3 and 5 cm lesions are still visible, but are reduced by 1/3. The patient is followed up.

20 **Example 9**

A 40 year old patient presents with a malignant tumor in the right tonsillar area, 2 cm in diameter, with a lymphnode of 3 cm of diameter in the right cervical area. 1 g of composition is injected in each lesion. The composition is made from a poly(ortho)ester (POE) polymer,, 200 mg of 2-FDG (200 mg per g of POE), fluoroacetate attached to cholesterol (1-4 mg per g of POE), and 5 mg of dinitrophenol (5 mg per g of POE). The lesions disappear and the 25 patient is regularly followed for recurrence.

Example 10

A 50 y old woman presents with a single 50 cc recurrent mass in the pelvis after multiple 30 chemotherapy sessions received for an ovarian cancer. The mass is accessible for punctures through the perineal area. Surgical debulking is not possible because of the previous multiple surgical interventions and the presence of adhesions. A Positron Emission Tomography coupled with CT scan examination (PET CT) using radioactive 18-F deoxyglucose shows high tumour uptake with a standard uptake value (SUV) of 11.

The tumour is accurately implanted under open magnetic resonance (MR). 4 rods of 1 mm of lactide-capronolactone, containing each 15 mg of rhodamine and 1 mg of Fluoroacetate are implanted at the 4 angles of the lesion. In addition a catheter perforated with 6 small holes of 50 microns of diameter is placed in the center of the tumour. The catheter exits the tumour
5 and is covered with small plaster for 2 weeks. After 2 weeks, the tumour is filled with rhodamine and Fluoroacetate, and the surviving cells are avid for glucose. Up to 50 mCi of cumulated 18FDG are injected in the central axis of the tumour, through the 6 lateral holes of the catheter. Each injection procedure lasts for a few milli-seconds, which allows to keep the injected substance inside the tumour volume and to progressively saturate the tumour
10 volume, at pressures of 2000-5000 atmospheres. Injections may be fractionated, for instance every day or every second day. The treatment effects may be monitored "live" under a PET CT machine. Once PET CT shows that the tumour is full of 18 FDG, the injections may be stopped and be performed on the next days. The dose delivered during the first hour to the tumour mass may be in the range of 70 Gy, which is a therapeutic dose. The aim is to keep
15 the activity inside the tumour volume only, as 18FDG is actively taken up by proliferating cells and much less by healthy tissues. The tumour shrinks in the following weeks, the CA-125 value drops and the lesion remains 1 cm in diameter during follow-up.

Example 11

20 A 40 y old patient presents with a basal carcinoma of the skin, 1 cm in diameter, located in the right side of his thorax. A hydrogel foil, 3 mm thick, 2.5 cm in diameter containing 10 mg per square cm of oxamate, 1 mg per square cm of dinitrophenol and 0.1 mg per square cm of fluoroacetate is deposited on the lesion and fixed with a translucent adhesive bandage. The foil is changed every day during 2 weeks. At 6 weeks the patient is examined showing
25 only a scar at the place where the lesion was present. The patient is followed up.

CLAIMS

1. Composition comprising one or more inhibitors of the citric acid, TCA, cycle, one or more inhibitors of glycolysis, one or more inhibitors of oxidative phosphorylation and one or more slow release agents.

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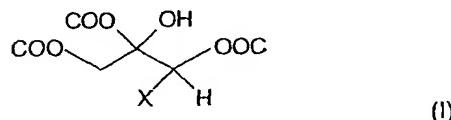
2. Composition according to claim 1, wherein said TCA cycle inhibitor is an inhibitor of one or more of pyruvate dehydrogenase, citrate synthase, aconitase, isocitrate lyase, alpha-ketoglutarate dehydrogenase complex, succinyl CoA synthetase, succinate dehydrogenase, fumarase, malate synthase, glutaminase and pyruvate dehydrogenase complex.

10

3. Composition according to claims 1 or 2, wherein said TCA cycle inhibitor is any of arsenite, hypoglycin A, methylenecyclopropylacetic acid, alloxan, PNU, p-benzoquinone, fluoroacetate, halogenated acetates (iodo-, bromo-, chloro-acetate), halogenated acetyl-CoA (fluoroacetyl-CoA, bromoacetyl-CoA, chloroacetyl-CoA, iodoacetyl-CoA), halogenated 15 crotonate (fluoro-, iodo-, bromo-, chloro-crotonate), halogenated ketone bodies, (chloro-, fluoro-, bromo-, iodoaceto-acetate, fluoro-, chloro-, bromo-, iodo-butyrate, fluoro-, chloro-, bromo-, iodo-acetone), halogenated oleate (iodo, bromo, chloro, fluoro-oleate), halogenated citrate, halogenated citrate 2R, 3R isomer (fluoro-, bromo-, chloro-, iodo-citrate), dichlorovinyl-cysteine, halogenated aminoacids, malonate, pentachlorobutadienyl-cysteine, 20 2-bromohydroquinone, 3-nitropropionic acid, cis-crotonalide fungicides, glu-hydroxyoxamate, p-chloromercuriphenylsulphonic acid, L-glutamate gamma-hydroxamate, p-chloromercuriphenylsulphonic acid, acivicin (alpha-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid , halogenated glutamine (fluoro, iodo, chloro, bromo-glutamine), or halogenated glutamate (fluoro, iodo, chloro, bromo-glutamate).

25

4. Composition according to claim 1 or 2, wherein said TCA cycle inhibitor is a compound of formula (I):



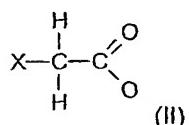
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where X is halide, a sulfonate, a carboxylate, an alkoxide, an amine oxide or OH.

5. Composition according to claim 4, where in formula (I):

- a halide is selected from the group consisting of: fluoride, bromide, chloride, and iodide,
- a sulfonate may be selected from the group consisting of: triflate, mesylate and tosylate,
- 5 - a carboxylate may be selected from the group consisting of: methoxylate and ethyloxylate,
- an alkoxide may be selected from the group consisting of: methoxide and ethoxide,
- an amine oxide is dimethylamine oxide, and
- 10 - where the stereochemistry is 2R, 3R,

6. Composition according to claims 1 or 2 wherein said TCA cycle inhibitor is a compound of formula (II):



15

where X is a halide, a sulfonate, a carboxylate, an alkoxide, an amine oxide, or an OH.

7. A composition according to claim 6, where in formula (II):

- the halide is selected from the group consisting of: fluoride, bromide, chloride, and iodide,
- 20 - the sulfonate is selected from the group consisting of: triflate, mesylate and tosylate,
- the carboxylate is selected from the group consisting of: methoxylate and ethyloxylate,
- 25 - the alkoxide is selected from the group consisting of: methoxide and ethoxide, and
- the amine oxide is dimethylamine oxide.

8. Composition according to claims 1 or 2 wherein said TCA cycle inhibitor is any of p-benzoquinone, thiaminase, fluoroacetamide, halogenated ketone bodies, chloroacetoacetate, fluoroacetoacetate, fluorohydroxybutyrate, chlorohydroxybutyrate, bromohydroxybutyrate), halogenated acetic acid, chloracetic acid, 6-diazo-5-oxo-L-norleucine (DON).

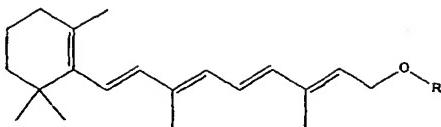
9. Composition according to claims 1 or 2, wherein said TCA cycle inhibitor is any of fluoroacetate, arsenite, acetoacetate, and betahydroxy butyrate.
- 5 10. Composition according to any of claims 1 to 9, wherein said TCA cycle inhibitor when comprising a halogen atom, comprises a halogen radioisotope in place of a stable halogen atom.
- 10 11. Composition according to any of claims 1 to 10, wherein said inhibitor of glycolysis inhibits at least one enzyme from the group consisting of hexokinase, glucokinase, phosphoglucose isomerase, phosphofructokinase, aldolase, triose phosphate isomerase, glyceraldehyde 3-phosphate dehydrogenase, phosphoglycerate kinase, phosphoglyceromutase, enolase, pyruvate kinase, and lactate dehydrogenase.
- 15 12. A composition according to any of claims 1 to 11, wherein said inhibitor of glycolysis is a hexose sugar modified by removal of the hydroxyl group or by the substitution of the hydroxyl group with halogen atom or thiol at:
- C6,
 - C1 or C2 or C5,
 - 20 - C3 and/or C4, and/or
 - C2 or C3.
13. A composition according to any of claims 1 to 11, wherein said inhibitor of glycolysis is any of 6-deoxy-6-fluoro-D-glucose, 6-deoxy-6-bromo-D-glucose, 6-deoxy-6-chloro-D-glucose, 6-O-methyl-D-glucose, 6-thio-D-glucose, 6-deoxy-D-glucose, C-6 modified or blocked derivatives of other hexose ring pyranoses, mannopyranoses, galactopyranoses, 6-deoxy-6-fluoro-D-glucose, 6-deoxy-6-bromo-D-mannose, 6-deoxy-6-chloro-D-mannose, 6-deoxy-6-fluoro-D-galactose, 6-deoxy-6-chloro-D-galactose, 6-deoxy-6-iodo-D-galactose, 6-deoxy-6-bromo-D-galactose, halogenated C-6 sugars gluconolactones, glucuronic acid, glucopyranoside, and their phosphate derivatives, glucuronides with halogenated glycosides at the C-1 position, C-2 substituted D-hexoses, 2-deoxy-2-halogeno-D-hexoses, 2-deoxy-2-fluoro-D-glucose, 2-chloro-2-deoxy-D-glucose, 2-bromo-D-glucose, 2-iodo-D-glucose, 2-deoxy-2,2-difluoro-D-arabino-hexose, 2-deoxy-2-fluoro-D-mannose, 2-deoxy-D-arabino-hexose, 2-Deoxy-2-fluoro-D-galactose, 1,6-anhydro-2-deoxy-2-fluoro-beta-D-glucopyranose, 30 35 1-6-anhydrosugar, 2-amino-2-deoxy-D-glucose, glucose amine, 2-amino-2-deoxy-D-

galactose, galactosamine, 2-amino-2-deoxy-D-mannose, mannosamine, 2-deoxy-2-fluoro-D-mannose, 2-deoxy-2-fluoro-D-galactose, 2-deoxy-D-arabino-hexose, 2-deoxy-2,2-difluoro-D-arabino-hexose, 2-deoxy-2-fluoro-D-glucose 1-Phosphate, 2-deoxy-2-fluoro-D-glucose 6-P, 2-deoxy-2-fluoro-D-glucose 1,6 biphosphate, 2-deoxy-2-fluoro-D-mannose 1-P, 2-deoxy-2-fluoro-D-mannose 6-P, 2-deoxy-2-fluoro-D-mannose 1,6-biphosphate, nucleotide diphosphate, uridine di-P, 1-2 deoxy-2-fluoro-D-glucose, C-2-halogen substituted, and NH3 substituted derivatives of D-Glucose 6-phosphate, 2-deoxy-2-fluoro-2-D-glucose-6-phosphate, 2-chloro-2-deoxy-D-glucose-6-phosphate, 2-deoxy-D-arabino-hexose-6-phosphate, D-glucosamine-6-phosphate, 2-deoxy-2-fluoro-2-D-mannose-6-P, and any known derivatives, C-2 halogenated derivatives of hexose ring pyranoses, mannopyranoses, galactopyranoses, C-2-deoxy-2- fluoropyranoses, and any derivative, C-2 halogenated sugars derivatives, C-2 fluoro-, bromo-, chloro-, or iodo-sugars derivatives, fluoro, bromo, chloro, or iodo C-2 sugars derivatives, gluconolactones, glucuronic acid, glucopyranoside, and their phosphate derivatives, sugars modified at C-1 or C-5 by replacement of hydroxyl by fluorine or deoxygenation or replacement by a sulfur group, glucosyl fluoride, 1-deoxy-D-glucose, 5-thio-D-glucose, 3-deoxy or 3-fluoro-D-glucose or 4-deoxy or 4-fluoro-D-glucose, 2-fluoro- or 2-iodo-, or 2-thio-, or 2-methoxy- or 3-fluoro-, or 3, 3 difluoro-, 3-iodo-, or 3-carboxylo-, or 3-thio-glyceraldehydes or glycerates, 3-fluoro-2-phosphoglycerate, phosphothioesters or other phosphor modified analogs, mannoheptulose mannoheptose, glucoheptose, N-acetylglucosamine, 6-aminonicotinamide acidosis-inducing agents, 2-deoxy-2-fluoro-D-glucose, citrate and halogenated derivatives of citrate, fructose 2,6-bisphosphate, bromoacetylethanolamine phosphate analogues, N-(2-methoxyethyl)-bromoacetamide, N-(2-ethoxyethyl)-bromoacetamide, N-(3-methoxypropyl)-bromoacetamide), iodoacetate, pentalenolactone, arsenic, 1,1-difluoro-3-phosphate-glycerol, oxamate, 2-fluoro-propionic acid or its salts, 2,2-difluoro-propionic acid, 3-halopropionic acid, or 2-thiomethylacetic acid.

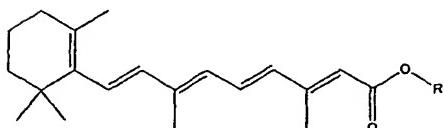
14. A composition according to any of claims 1 to 11, wherein an inhibitor of glycolysis is any of 2FDG, oxamate or iodoacetate.
- 30 15. A composition according to any of claims 1 to 14, wherein said glycolysis inhibitor, when comprising a halogen atom, comprises a halogen radioisotope in place of a stable halogen atom.

16. A composition according to any of claims 1 to 15, wherein said oxidative phosphorylation inhibitor is an inhibitor of one or more of complex I (NADH coenzyme Q reductase), II (succinate-coenzyme Q reductase), III (coenzyme Q cytochrome C reductase), IV (cytochrome oxydase), and V (F0-F1, ATP synthase).
- 5 17. A composition according to any of claims 1 to 16, wherein said oxidative phosphorylation inhibitor is any of rotenone, amytal, 1-methyl-4-phenylpyridinium, paraquat, myxothiazol, antimycin A, ubisemiquinone, cytochrome C, 4,6-diaminotriazine derivatives, cyanide, hydrogen sulfide, azide, formate, phosphine, carbon monoxide, 4'-demethyl-epipodophyllotoxin thenylidene glucoside, tritylthioalanine, carminomycin, piperazinedione, dinitrophenol, dinitrocresol, 2-hydroxy-3-alkyl-1,4-naphtoquinones, apoptolidin aglycone, oligomycin, ossamycin, clofazimine cytovaricin, naphtoquinone derivatives, dichloroallyllawsone, lapachol, rhodamine, rhodamine 123, rhodamine 6G, carbonyl cyanide p-trifluoromethoxyphenylhydrazone, cyhexatin, dichlorodiphenyltrichloroethane (DDT), chlordecone, arsenate, pentachlorophenol, benzonitrile, thiadiazole herbicides, salicylate, cationic amphiphilic drugs, amiodarone, perhexiline, gramicidin, calcimycin, pentachlorobutadienyl-cysteine, trifluorocarbonylcyanide phenylhydrazone, atracyloside, lysophospholipids, n-ethylmaleimide, mersanyl, or p-benzoquinone.
- 10 18. A composition according to any of claims 1 to 16, wherein said inhibitor of oxidative phosphorylation is any of rhodamine, rhodamine 6G, rhodamine 123, dinitrophenol, or rotenone.
- 15 19. A composition according to any of claims 1 to 18, wherein said oxidative phosphorylation inhibitor, when comprising a halogen atom, comprises a halogen radioisotope in place of a stable halogen atom.
- 20 20. A composition according to any of claims 1 to 19, wherein the oxidative phosphorylation inhibitor in an amount such that the concentration of inhibitor delivered to a subject is between 0.01 and 2 g/kg of tumour or of treated mass.
- 25 21. A composition according to any of claims 1 to 20, further comprising one or more pyrophosphates.

22. A composition according to claim 21, wherein said pyrophosphate is one or more of sodium pyrophosphate, potassium pyrophosphate, calcium pyrophosphate.
23. A composition according to claims 21 or 22, wherein said pyrophosphate is administered
5 simultaneous, separate or sequentially in respect of the inhibitors.
24. A composition according to any of claims 1 to 23, further comprising one or more imaging agents.
- 10 25. A composition according to any of claim 24, wherein said imaging agent is any of poly(ortho)ester, magnesium alloy powder, metallic powder, tantalum powder, biocompatible metal powder, iridium powder, or micro-bubbles.
- 15 26. A composition according to any of claims 1 to 25, wherein said slow release agent is any of poly(glycolic) acid, poly(lactic acid) or in general glycolic- and lactic acid based polymers, copolymers, poly caprolactones and in general, poly hydroxyl alkanoates poly(hydroxy alcanoic acids), Poly (ethylene glycol), poly vinyl alcohol, poly (orthoesters), poly (anhydrides), poly (carbonates), poly amides, poly imides, poly imines, poly (imino carbonates), poly (ethylene imines), polydioxanes, poly oxyethylene (poly ethylene oxide),
20 poly (phosphazenes), poly sulphones, lipids, poly acrylic acids, poly methylmethacrylate, poly acryl amides, poly acrylo nitriles (Poly cyano acrylates), poly HEMA, poly urethanes, poly olefins, poly styrene, poly terephthalates, poly ethylenes, poly propylenes, poly ether ketones, poly vinylchlorides, poly fluorides, silicones, poly silicates (bioactive glass), siloxanes (Poly dimethyl siloxanes), hydroxyapatites, poly aminoacids (natural and non
25 natural), poly β -aminoesters, albumines, alginates, cellulose / cellulose acetates, chitin / chitosan, collagene, fibrine / fibrinogen, gelatine, lignine, protein based polymers, Poly (lysine), poly (glutamate), poly (malonates), poly (hyaluronic acids), Poly nucleic acids, poly saccharides, poly (hydroxyalkanoates), poly isoprenoids, starch based polymers, copolymers thereof, linear, branched, hyperbranched, dendrimers, crosslinked, functionalised derivatives
30 thereof, hydrogels based on activated polyethyleneglycols combined with alkaline hydrolyzed animal or vegetal proteins.
27. A composition according to any of claims 1 to 26, wherein said composition further comprises magnesium alloys.



(IV)



(V)

28. A composition according to any of claims 1 to 27, wherein at least one of said inhibitors is coupled to solubilising agent.
29. A composition according to claim 28, wherein at least one said solubilising agent is cholesterol or derivative thereof.
30. A composition according to claim 29, wherein said cholesterol derivatives are any of cholesteryl-3-betahydroxybutyrate, cholesteryl-halogenated butyrate, cholesteryl-halogenated acetate, cholesteryl-halogenated aceto-acetate, cholesteryl-halogenated acetamide, cholesteryl-halogenated crotonate, cholesteryl-halogenated acetone, cholesteryl-halogenated citrate, or cholesteryl-halogenated oleate.
31. A composition according to claim 28, wherein at least one said solubilising agent is vitamin A or derivative thereof.
32. A composition according to claim 31, wherein derivative of vitamin A is formula (IV) or (V):
- wherein R is selected from the group consisting of betahydroxybutyrate, halogenated butyrate, halogenated acetate, halogenated aceto-acetate, halogenated acetamide, halogenated crotonate, halogenated acetone, halogenated citrate, and halogenated oleate.
33. A composition according to any of claims 1 to 32, wherein at least one of said inhibitors is present in micro-capsule and/or nano-capsule.

34. A composition according to claim 33, wherein nano-capsule is any of copolymer poly(ethylene oxide) with poly(L-Lactic acid) or with poly(beta-benzyl-L-aspartate), copolymer with poly(lactide-co-glycolide)-[(propylene oxide)-poly(ethylene oxide)], polyphosphazene derivatives, poly(ethylene glycol) coated nanospheres, poly(isobutylcyanoacrylate) nanocapsules, poly(gamma-benzyl-L-glutamate)/(poly(ethylene oxide)), chitosan-poly(ethylene oxide) nanoparticles, nanoparticles where said inhibitor is prepared using *o*-carboxymethylate chitosan as wall forming material, or solid lipid nanospheres (SLN).
- 10 35. A composition according to claim 33, wherein micro-capsule is any of multiporous beads of chitosan, coated alginate microspheres, N-(aminoalkyl) chitosan microspheres, chitosan/calcium alginate beads, poly(adipic anhydride) microspheres, gellan-gum beads, poly(D, L-lactide-co-glycolide) microspheres, alginate-poly-L-lysine microcapsules, crosslinked chitosan microspheres, chitosan/gelatin microspheres, crosslinked chitosan 15 network beads with spacer groups, 1,5-diozepan-2-one microspheres, D,L-dilactide microspheres, triglyceride liposomes, polyelectrolyte complexes of sodium alginate chitosan, polypeptide microcapsules, or albumin microspheres.
- 20 36. Composition according to any of claims 1 to 35, wherein said composition is part of a solid wall composition.
37. Composition according to claims 36, wherein said solid wall composition is a capsule of suitable size and shape for administration using a needle, said capsule filled with composition.
- 25 38. Composition according to claim 37, wherein a wall of said capsule comprises gelatin.
- 30 39. Composition according to claim 36, wherein said solid wall composition is a solid state bioabsorbable structure of suitable size and shape for administration using a needle, said structure impregnated with composition.
40. Composition according to any of claims 39, wherein said solid state bioabsorbable structure is seed-shaped, rod-shaped, or tube-shaped.

41. Composition according to any of claims 1 to 40, wherein at least one said inhibitor is a derivative of the inhibitor which is a stereoisomer, tautomer, racemate, prodrug, metabolite, pharmaceutically acceptable salt, base, ester, structurally related compounds or solvate.
- 5 42. Composition according to any of claims 1 to 41, for the treatment of cellular proliferation, wherein said composition is administered into the proliferating cell mass.
- 10 43. Use of a composition according to any of claims 1 to 41, for the manufacture of a medicament for the treatment of cellular proliferation, wherein said composition is administered into the proliferating cell mass.
- 15 44. Use of a composition according to any of claims 1 to 41, for the manufacture of a medicament for sensitising a cellular proliferation to treatment by radiotherapy, wherein said composition is administered into the proliferating cell mass prior to radiotherapy.
- 20 45. Use of a composition according to any of claims 1 to 41, for the manufacture of a medicament for sensitising a cellular proliferation to treatment by chemotherapy, wherein said composition is administered into the proliferating cell mass prior to chemotherapy.
- 25 46. Use or composition according to claims 1 or 45, wherein said TCA cycle, glycolysis and oxidative phosphorylation inhibitors are administered separately, simultaneously or sequentially.
- 30 47. Use or composition according to any of claims 1 to 46, further combined with radiotherapy.
- 35 48. Use or composition according to any of claims 1 to 46, further combined with chemotherapy.
- 30 49. Use or composition according to any of claims 1 to 48, wherein said composition is administered by injection into a mass of proliferating cells.
- 35 50. Use or composition according to any of claims 1 to 48, wherein said composition is administered by infusion into a mass of proliferating cells.

51. Use or composition according to any of claims 1 to 48, wherein said composition is administered by high-pressure injection into a mass of proliferating cells.
52. Use or composition according to any of claims 1 to 48, wherein said composition is administered in the resection cavity or scar of a proliferating mass.
53. Kit comprising a composition comprising one or more inhibitors of the TCA cycle, one or more inhibitors of glycolysis and one or more inhibitors of oxidative phosphorylation.
- 10 54. A kit according to claim 53 wherein said composition is a composition as defined in any of claims 1 to 41.
55. A kit according to claims 53 or 54, further comprising a syringe.
- 15 56. A hydrogel comprising a) composition as defined in any of claims 1 to 35, and b) an activated polyethyleneglycol (PEG) combined with any of alkaline hydrolyzed soya solutions, animal or vegetal proteins, bovine serum albumin, soya globulin, casein, pea albumin, starch albumine, or ovalbumin.
- 20 57. A hydrogel according to claim 56 wherein a TCA inhibitor of the composition is present at a concentration of less than or equal to 0.1 mg per square cm of hydrogel and/or a glycolysis inhibitor of the composition is present at a concentration of less than or equal to 10 mg per square cm of hydrogel and/or an oxidative phosphorylation inhibitor of the composition is present at a concentration of less than or equal to 1 mg per square cm of hydrogel.
- 25 58. A use of a hydrogel according to claim 56 or 57 for treatment of superficial cell proliferation, such as basal carcinoma or a squamous cell carcinoma by application of the hydrogel to the surface of said proliferations.

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER				
A61K31/19 A61K31/352 A61P13/08 A61P35/00 A61K31/70 A61K45/06 A61K31/197 A61K31/06 A61K9/00				
According to International Patent Classification (IPC) or to both national classification and IPC				
B. FIELDS SEARCHED				
Minimum documentation searched (classification system followed by classification symbols)				
A61K A61P				
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched				
Electronic data base consulted during the international search (name of data base and, where practical, search terms used)				
EPO-Internal, BIOSIS, WPI Data, PAJ, EMBASE, CHEM ABS Data				
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<input checked="" type="checkbox"/> Further documents are listed in the continuation of box C.		<input checked="" type="checkbox"/> Patent family members are listed in annex.		
* Special categories of cited documents : 'A' document defining the general state of the art which is not considered to be of particular relevance 'E' earlier document but published on or after the international filing date 'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) 'O' document referring to an oral disclosure, use, exhibition or other means 'P' document published prior to the international filing date but later than the priority date claimed				
T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. *& document member of the same patent family				
Date of the actual completion of the international search		Date of mailing of the international search report		
26 January 2006		03/02/2006		
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European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl. Fax (+31-70) 340-3016		Pilling, S		

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